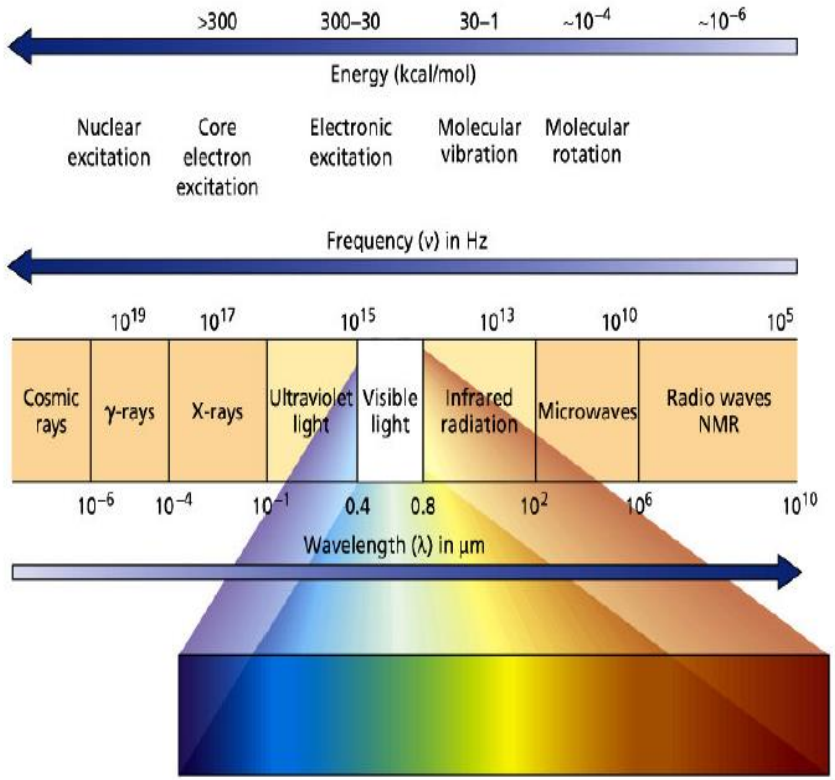


INTRODUCTION OF IR SPECTROSCOPY

- Infrared spectroscopy is an important analytical technique for determining the structure of both inorganic & organic compounds. It is also known as vibrational spectroscopy
- IR radiations lies in the wavelength range of 0.7 - 400 μm.
- IR spectroscopy is based upon selective absorption of IR radiations by the molecule which induces vibration of the molecules of the compound.
- IR instruments are of 2 types namely, dispersive instruments (spectrophotometers) and Fourier transform IR instrument.
- The radiation sources used are incandescent lamp, Nernst glower etc., and the detectors used are thermal and photon detectors.

NATURE OF IR SPECTRA

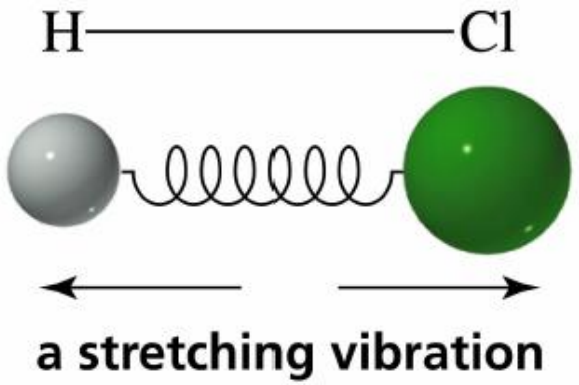
- IR spectrum is a graph of band intensities on ordinate versus position of band on abscissa.
- Band intensities can be given in terms of transmittance(T) or absorbance(A).
- Position of band can be expressed in terms of wave number (n) or wavelength(λ).
- In IR spectra, wave numbers (n) are used instead of wavelength (λ) for mentioning the characteristic peak as this unit has advantage of being linear with energy of radiation (E) .

$$E = h\,c/\lambda \quad \text{or,} \quad E = h\,c\,n$$

[$n = 1/\lambda$, c= velocity of light, h= Planck's constant]

PRINCIPLE OF IR SPECTROSCOPY

- When the energy in the form of IR is applied and if the applied IR frequency = Natural frequency of vibration, the absorption of IR takes place and a peak is observed.
- Molecules are excited to the higher energy state from the ground state when they absorb IR radiation.
- When a compound is exposed to IR radiation, it selectively absorbs the radiations resulting in vibration of the molecules of the compound, giving rise to closely packed absorption bands, called as IR absorption spectrum.
- The bands correspond to the characteristic functional groups and the bonds present in a chemical substance. Thus, an IR spectrum of a compound is considered as the fingerprint for its chemical identification.



CHANGE IN DIPOLAR MOMENT OF THE MOLECULES MUST OCCUR!

H—Cl

ACTIVE IR STRECHTING

O=C=O

H—H

INACTIVE IR STRECHTING

O=C=O

CRITERIA FOR A COMPOUND TO ABSORB IR RADIATION

1. Correct wave length of incident radiation

- A molecules absorbs radiation only when the frequency of the incident radiation is equivalent to the natural frequency of vibration of the part of the molecule.
- After absorption of the correct wave length of radiations, the molecule vibrates at increased amplitude due to absorbed IR energy.
- Example: HCl has natural vibrational frequency of 8.7×10^{13}/s (2890 cm-1). When HCl sample is exposed to IR radiations, only the radiations of frequency 8.7×10^{13}/s are absorbed and remaining are transmitted.

PEAK POSITION

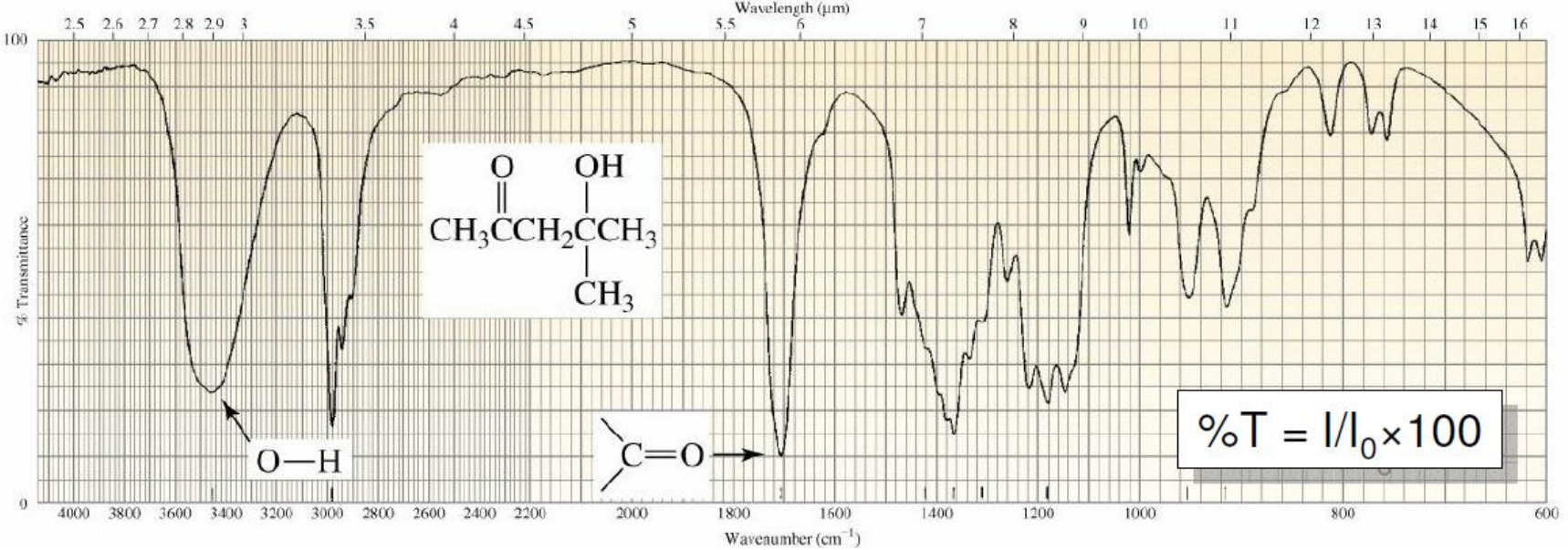
$$\tilde{\nu} = \frac{1}{2\pi c}\sqrt{\frac{k}{m^*}}$$

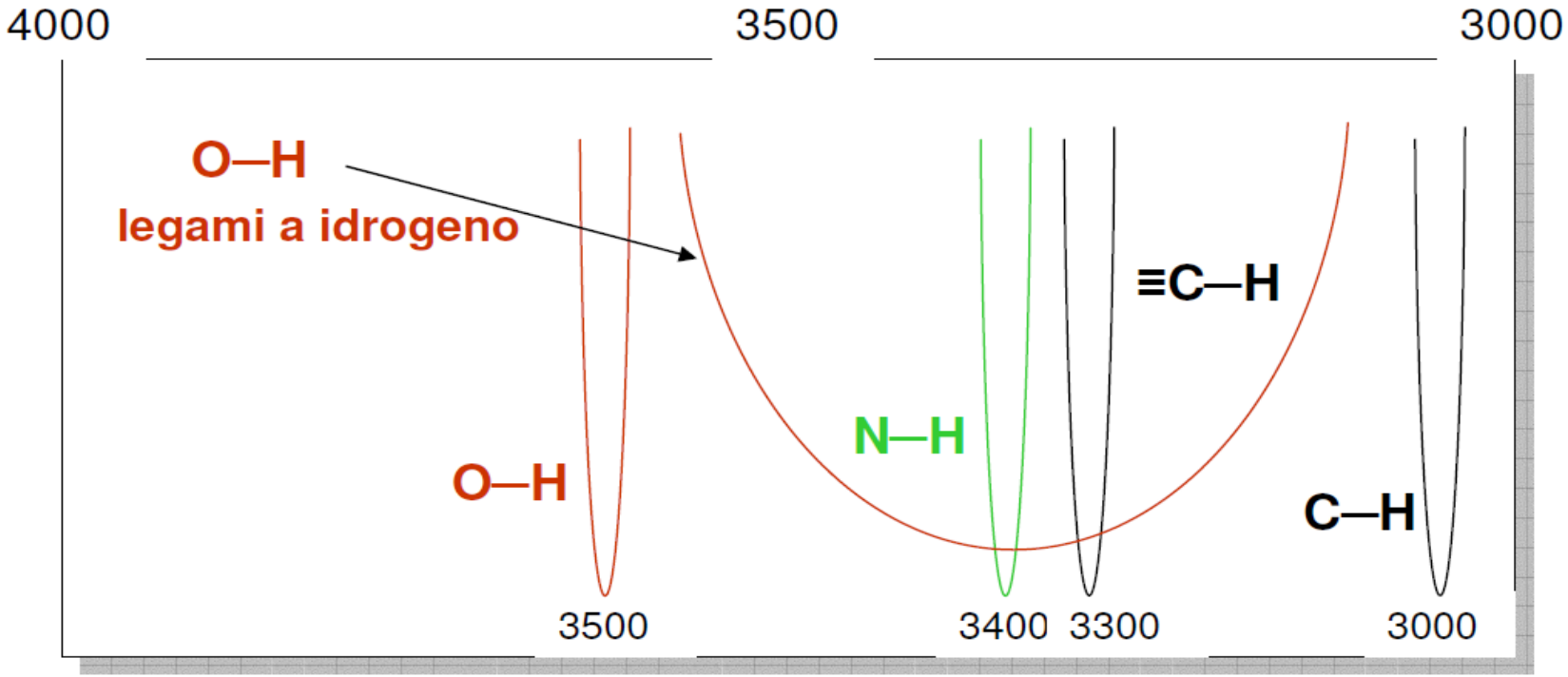
k = bond strenght

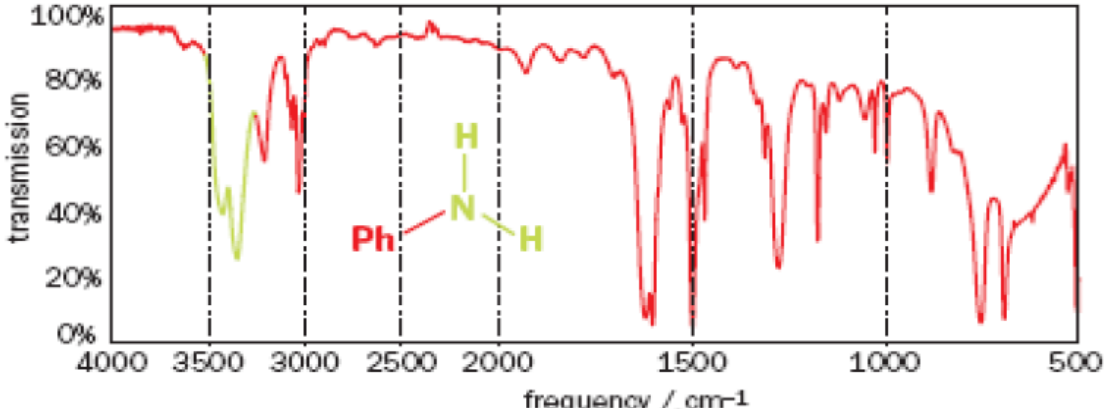
$m^* = m_A\, m_B / (m_A + m_B)$

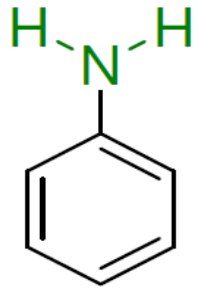
O–H > N–H > C–H

BOND STRENGHT

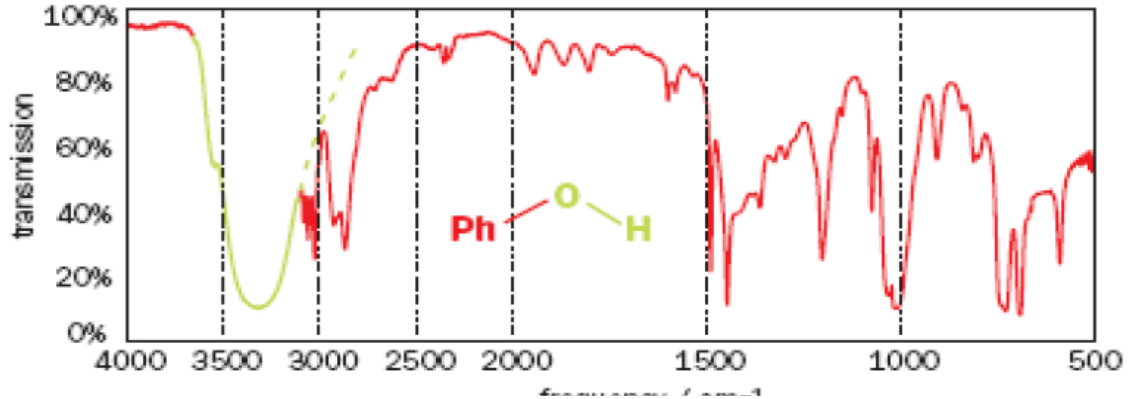


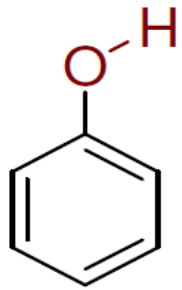




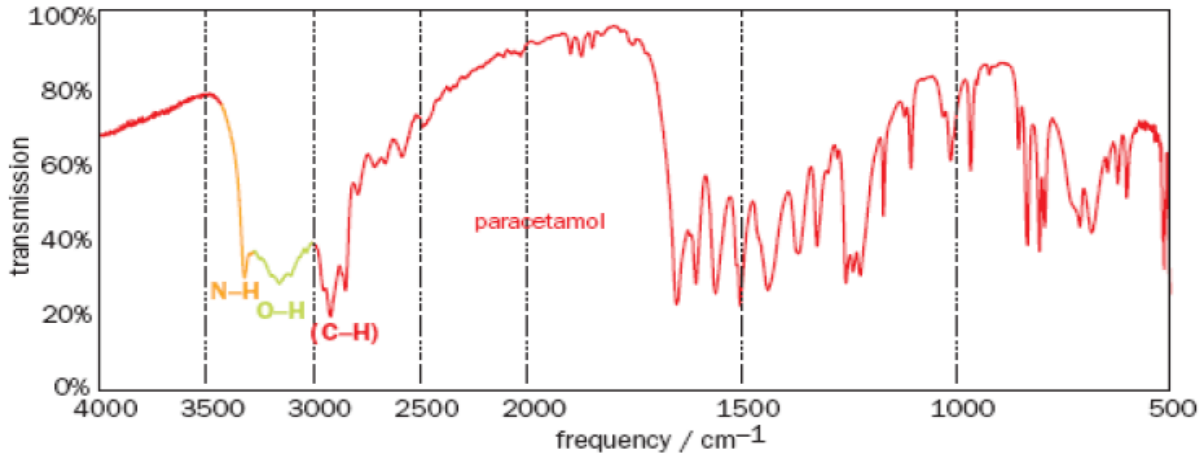


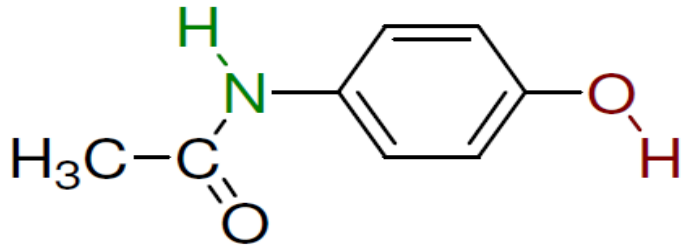
SIMMETRIC AND ASIMMETRIC STRETCHING





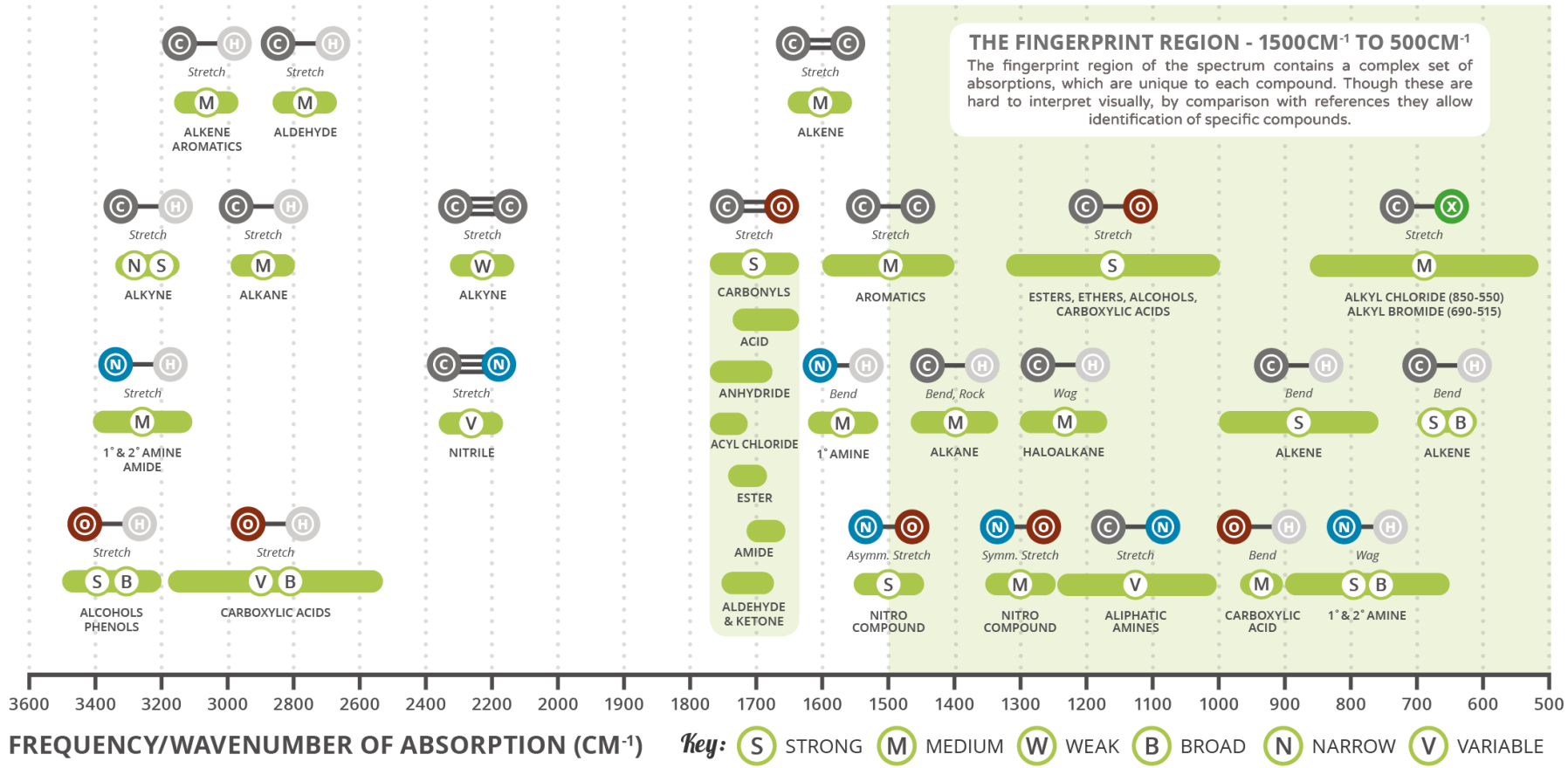
HYDROGEN BONDS



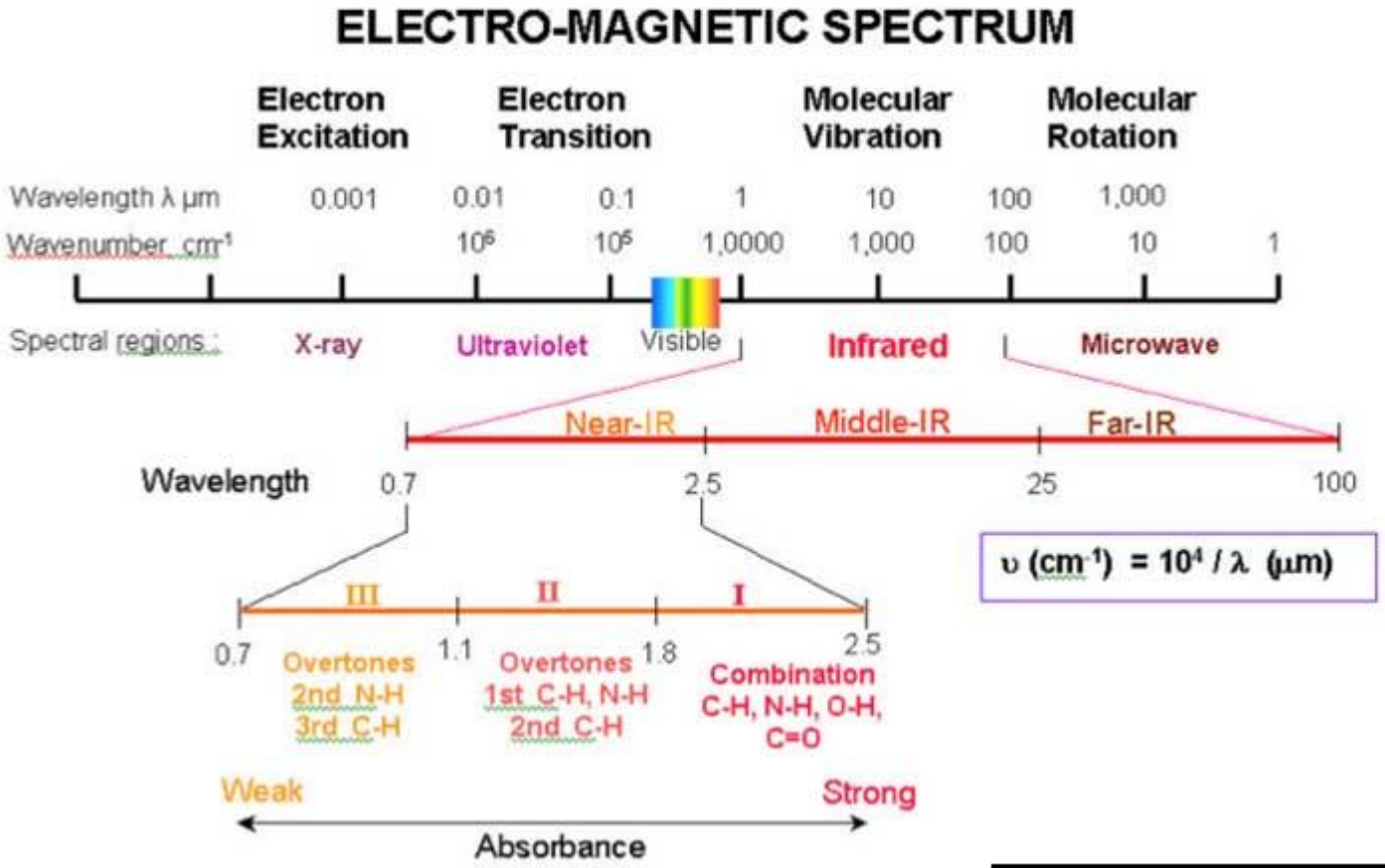


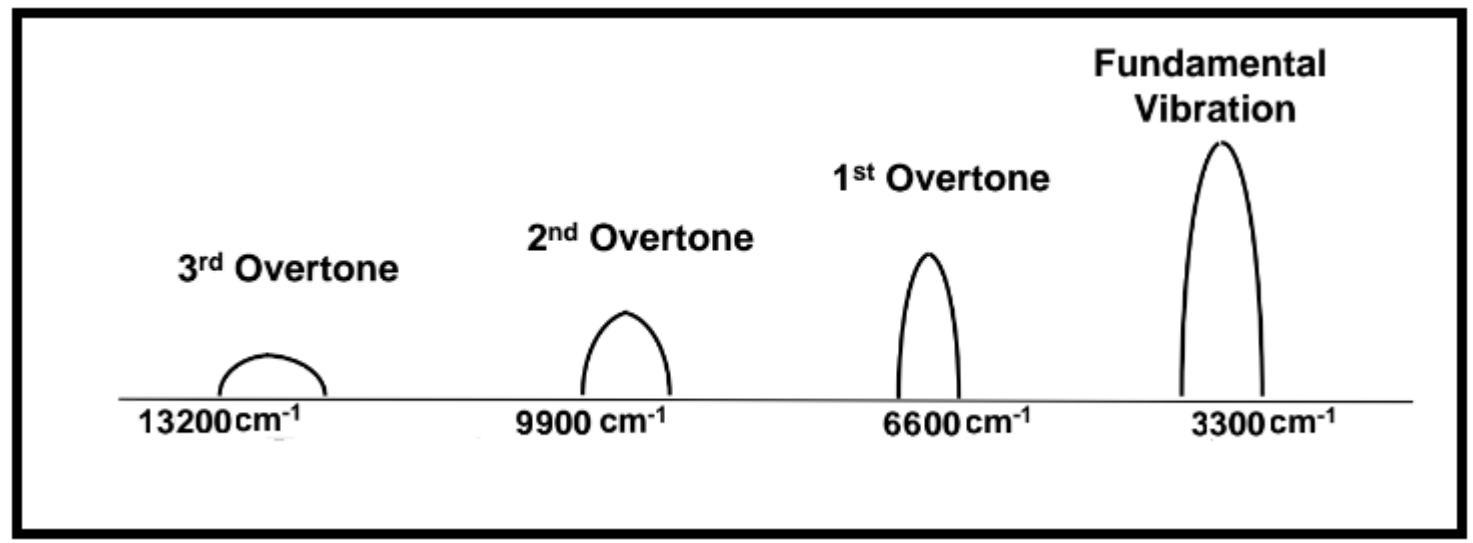
ANALYTICAL CHEMISTRY - INFRARED SPECTROSCOPY

Commonly referred to as IR spectroscopy, this technique allows chemists to identify characteristic groups of atoms (functional groups) present in molecules.

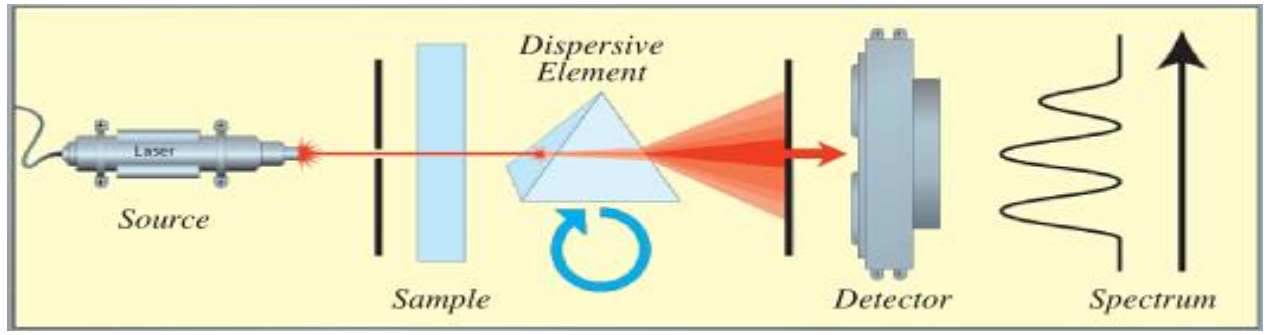


Infrared frequencies make up a portion of the electromagnetic spectrum. If a range of infrared frequencies are shone through an organic compound, some of the frequencies are absorbed by the chemical bonds within the compound. Different chemical bonds absorb different frequencies of infrared radiation. There are a number of characteristic absorptions which allow functional groups (the parts of a compound which give it its particular reactivity) to be identified. This graphic shows a number of these absorptions.

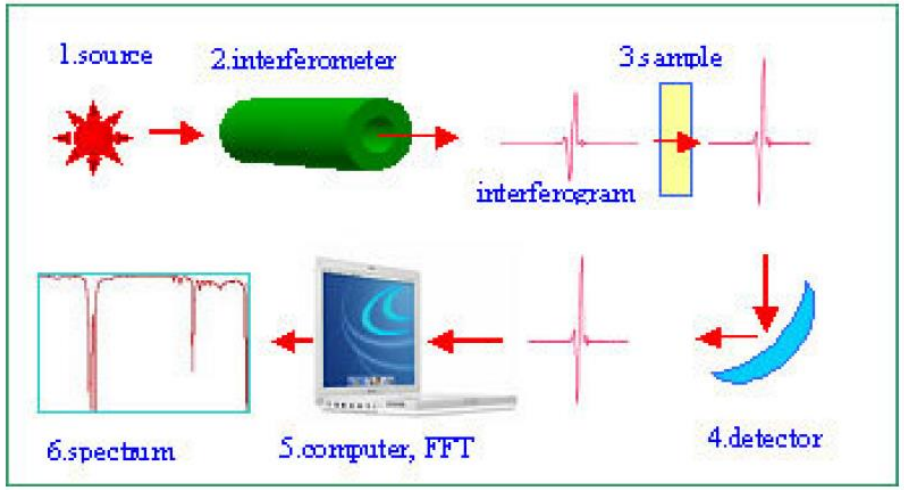

ELECTRO-MAGNETIC SPECTRUM
Electron Excitation
Electron Transition
Molecular Vibration
Molecular Rotation
Wavelength λ µm
0.001
0.01
0.1
1
10
100
1,000
Wavenumber, cm-1
10^6
10^5
1,0000
1,000
100
10
1
Spectral regions :
X-ray
Ultraviolet
Visible
Infrared
Microwave
Near-IR
Middle-IR
Far-IR
Wavelength
0.7
2.5
25
100
υ (cm-1) = 10^4 / λ (µm)
III
II
I
0.7
1.1
1.8
2.5
Overtones
2nd N-H
3rd C-H
Overtones
1st C-H, N-H
2nd C-H
Combination
C-H, N-H, O-H,
C=O
Weak
Strong
Absorbance

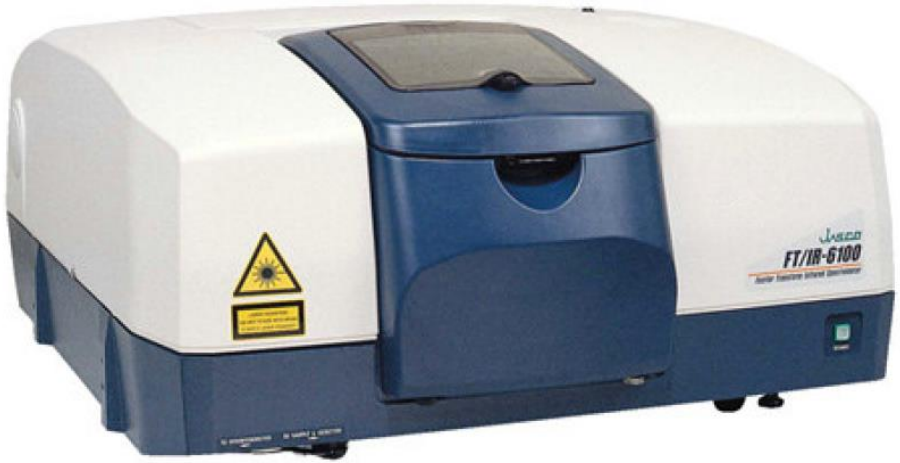

Fundamental Vibration
1st Overtone
2nd Overtone
3rd Overtone
13200 cm-1
9900 cm-1
6600 cm-1
3300 cm-1

SPETTROMETRI IR E FTIR (O FT-NIR)

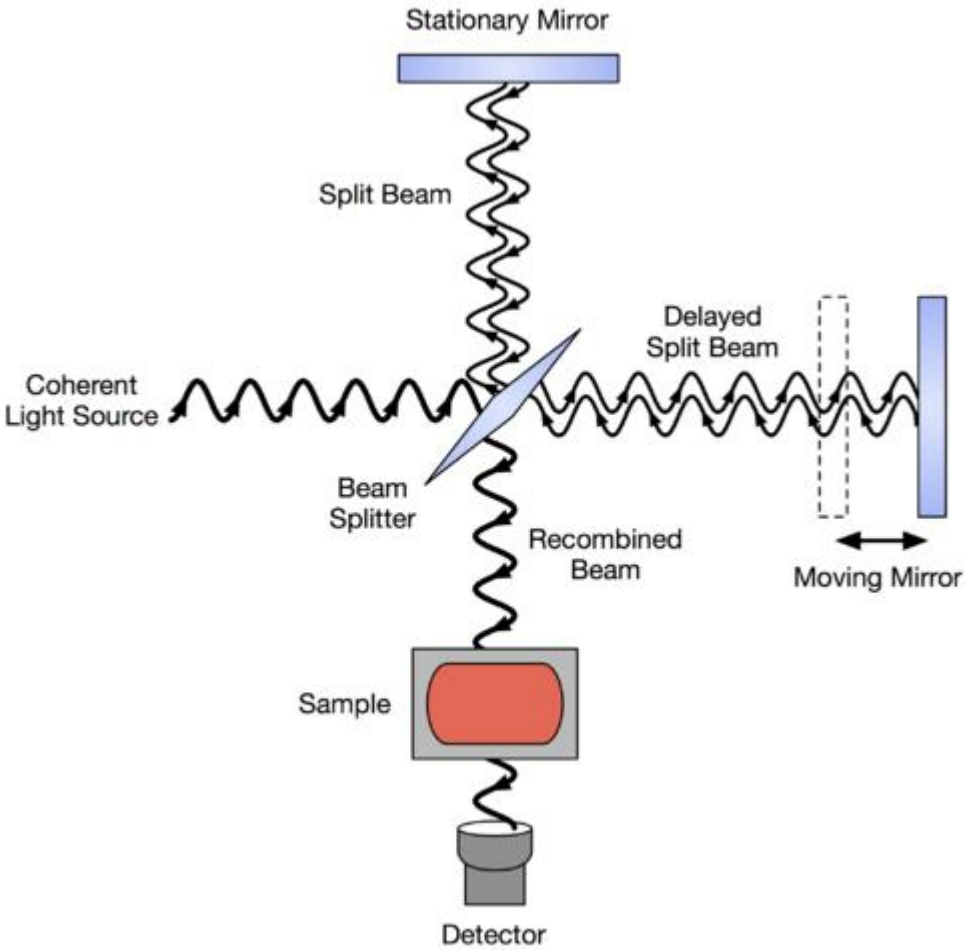


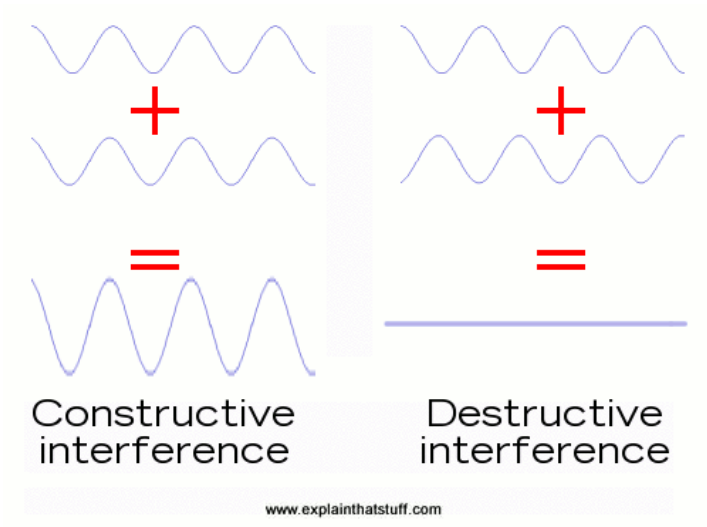
FT-IR

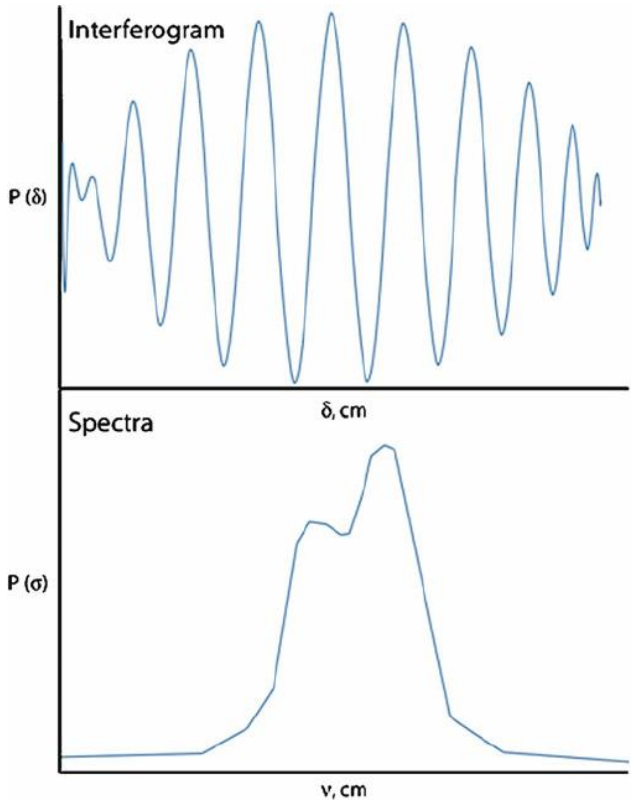




MICHELSON INTERFEROMETER

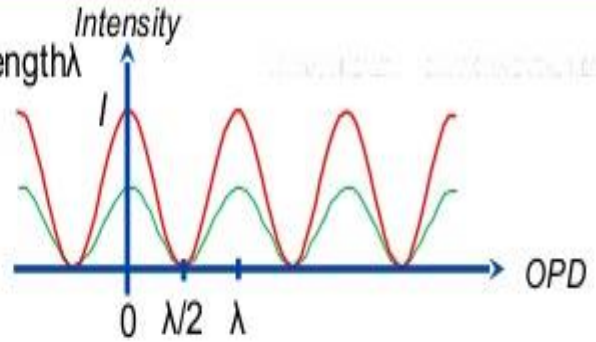




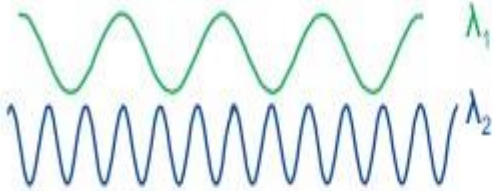


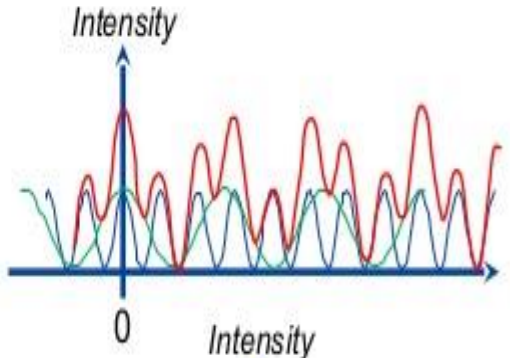
Light interference of multiple wavelengths(interferogram)

① monochromatic radiation of wavelengthλ

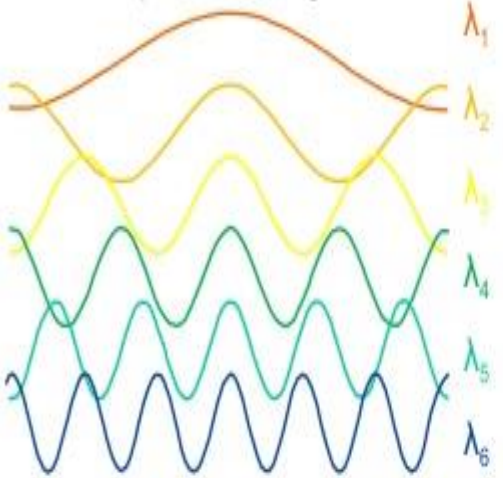


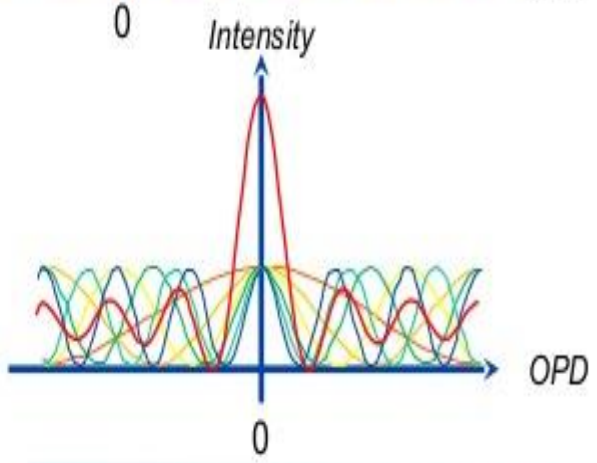
② two wavelengths radiation





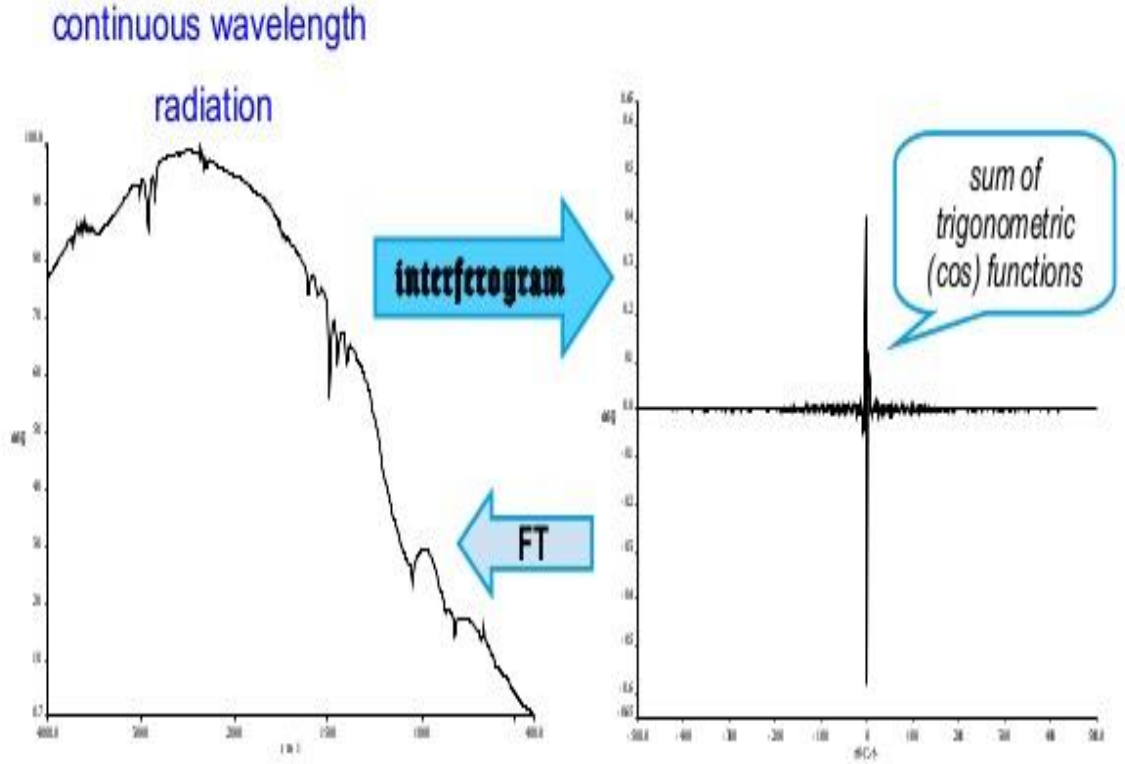
③ multiple wavelengths





intensity is strongest at 0 optical path difference

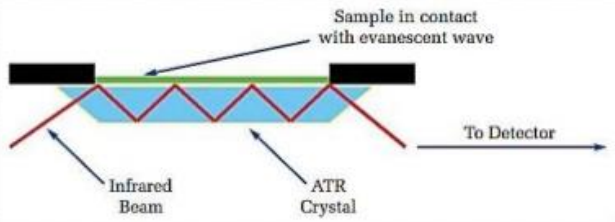
Energy in infrared region and interferogram



UNTREATED SAMPLES IN FTNIR

1. Attenuated Total Reflection

- The beam is directed onto optical dense crystal , *internal reflectance* create waves that extend to the sample in contact with crystal surface.



Analyze milk
using the Pearl FTIR

#SpectroscopyGuides

http://www.brukeroptics.cz/applications/food-feed-and-beverage/

http://galaxy-scientific.com/feed-and-feed-ingredients-analysis-ft-nir/

shelf life of Crescenza cheese

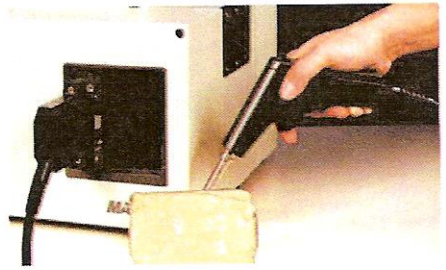


Figura 3.3: Fibra ottica a riflettanza diffusa posizionata direttamente sui campion

Resolution	16 cm^{-1}
Sample scan time	16 scans
Background scan time	4 scans
Save data from	12000 to 4400 cm^{-1}
Result spectrum	Absorbance
Source setting	NIR
Beam splitter	Quartz
Detector setting	TE-InGaAs [internal]
Scanner velocity	10 kHz

abella 3.1: Condizioni di acquisizione mediante fibra ottica a riflettanza diffusa

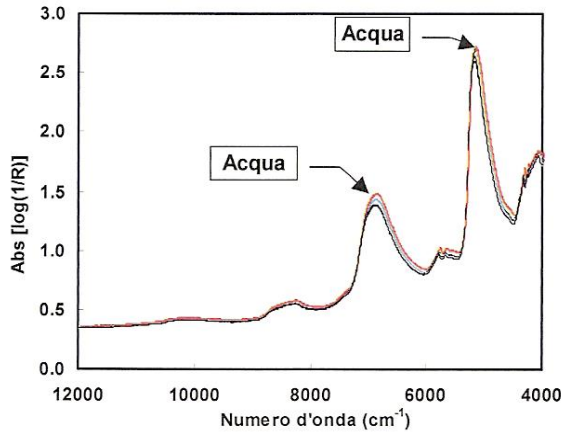
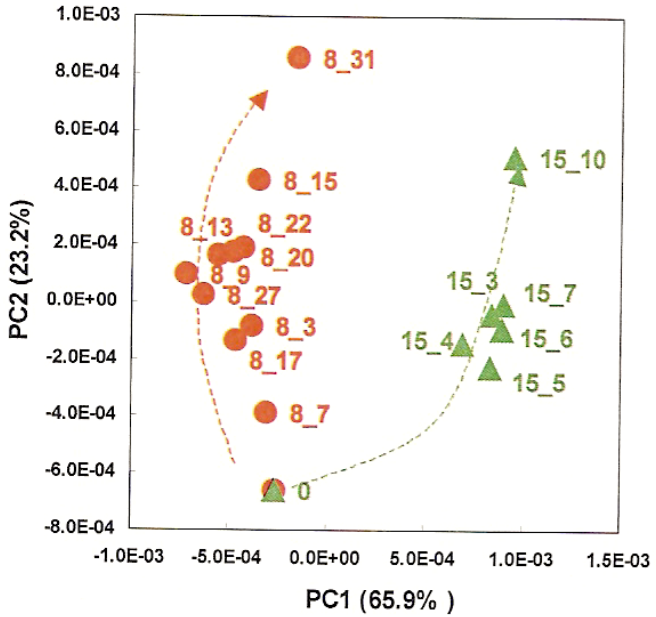


Figura 3.6: Esempio di serie di spettri di formaggio Crescenza acquisiti mediante fibra ottica

Optical fiber on the surface of the sample
5 spectra per sample, second derivative of the spectra used to ampilfy the difference
 PCA



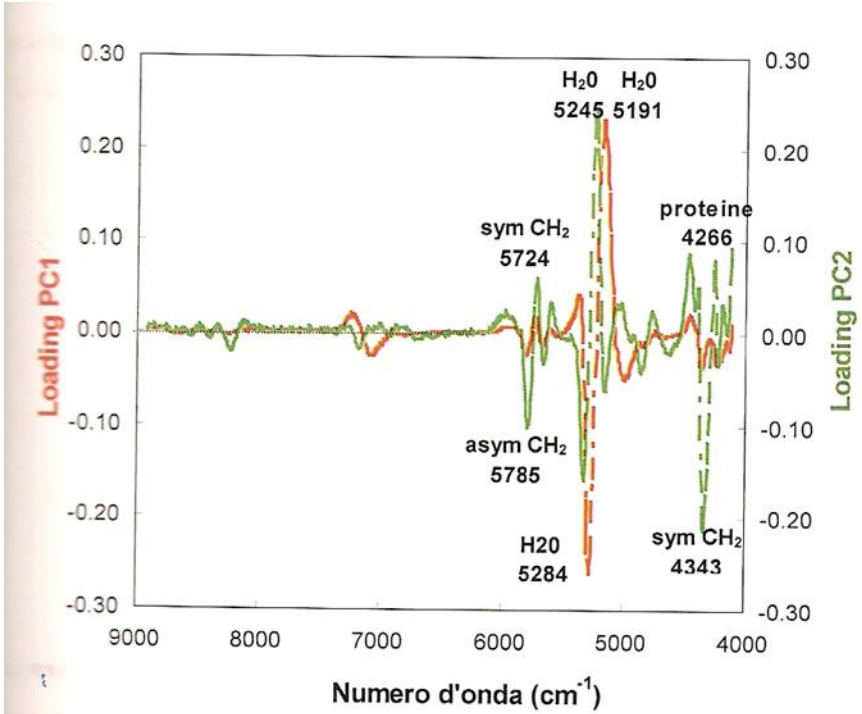
7: Score plot ottenuti dall'analisi della PCA applicata agli spettri trasformati in derivata seconda di formaggio Crescenza conservato a 8° e a 15 °C

The score plot shows difference in behviour of the samples at 2 different temperatures (8° and 15°), more evident at longer times.

The loading plot shows the role of each variable (wavelenght) with respect to the principal components

There are variations due to water and lipids . Using the second derivative spectra reorganisation of the proteic matrix is noticed.

The data (shelf life 6-7 days a 8-10°) comparable with chemical analysis



a 3.9: Loading plot ottenuti dall'analisi della PCA applicata agli spettri tra-nati in derivata seconda di formaggio Crescenza conservato a 8° e a 15 °C

Bakery products

Figura 4.1 : Allestimento sperimentale utilizzato per le prove di lievitazione

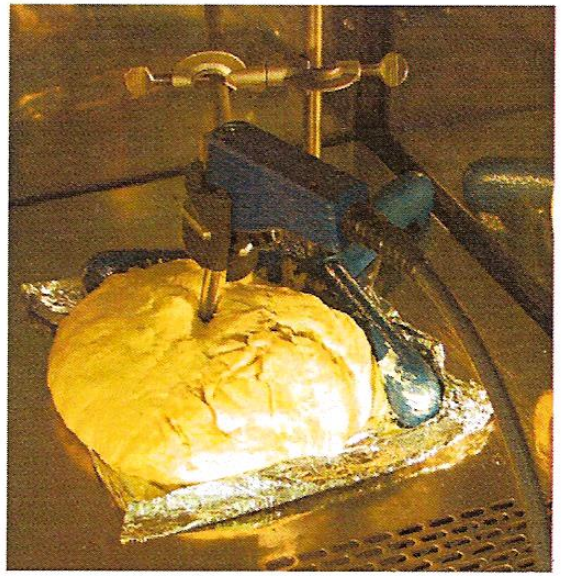


Figura 4.2 : Interfaccia fibra ottica e campione

Resolution	16 cm^{-1}
Sample scan time	64 scans
Background scan time	64 scans
Save data from	12000 to 4000 cm^{-1}
Result spectrum	Absorbance
Source Setting	NIR
Beam Splitter	Quartz
Detector Setting	TE-InGaAs
Scanner Velocity	10 kHz

Tabella 4.2 : Condizioni operative dello spettrometro MPA utilizzate nelle prove di lievitazione

Optical fiber in the centre
1 spectrum every 5 min per 180 min

BASELINE CORRECTION

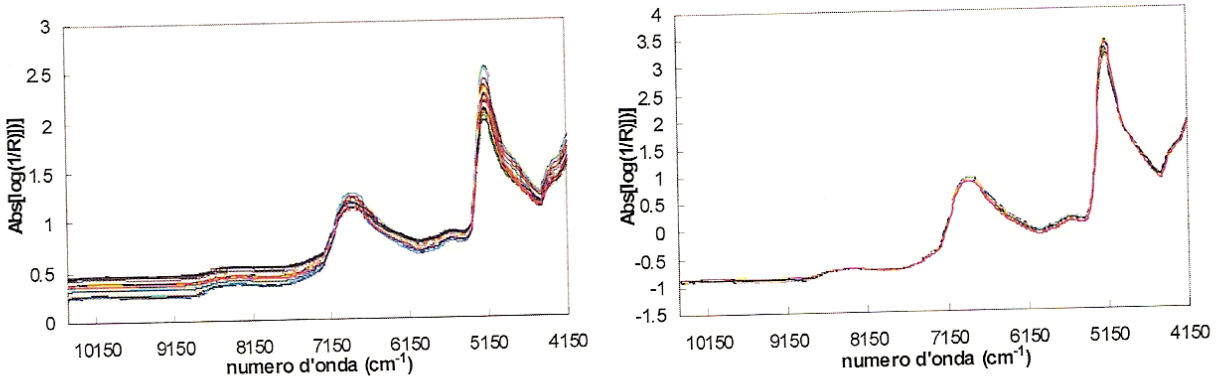


Figura 4.3: Serie di spettri raccolti durante la lievitazione prima (a sinistra) e dopo trattamento di standardizzazione SNV (a destra).

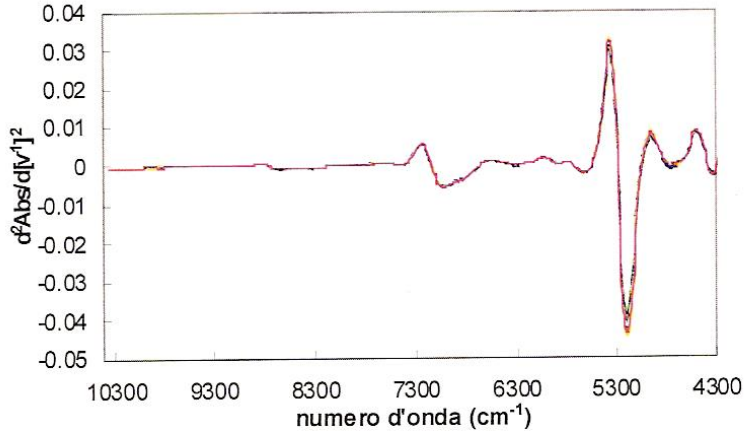


Figura 4.4: Serie di spettri in derivata seconda raccolti durante la lievitazione

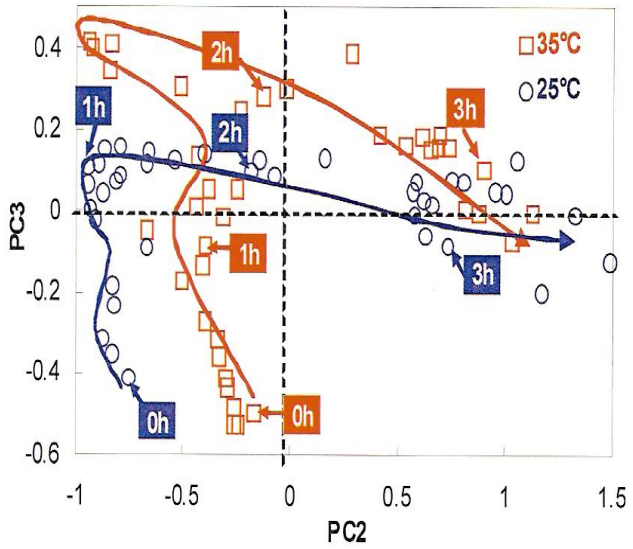


Figura 4.5: PCA Score Plot relativo ai test di lievitazione a 25 e 35°C (le etichette indicano la posizione dei campioni dopo 1,2 e 3 ore di lievitazione);

Score e loading plot PC1 explain 98% of the variance
Comparing PC2 e 3 evident contribution of water and starch (PC3)

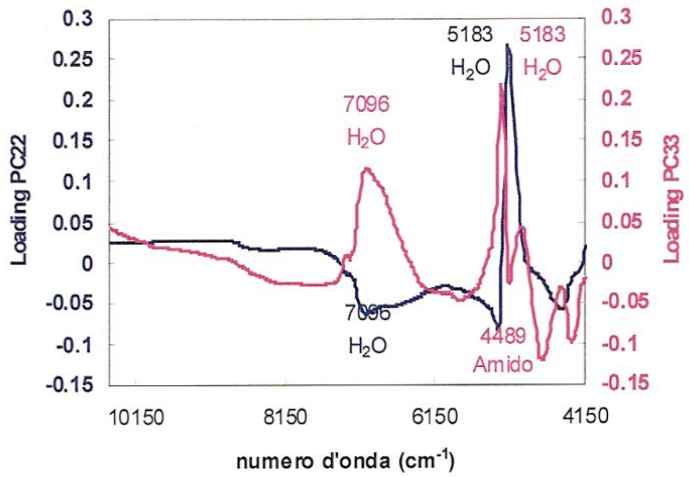


Figura 4.6 : PCA Loading Plot del test di lievitazione a 25°C inclusivo delle componenti chimiche coinvolte nella discriminazione

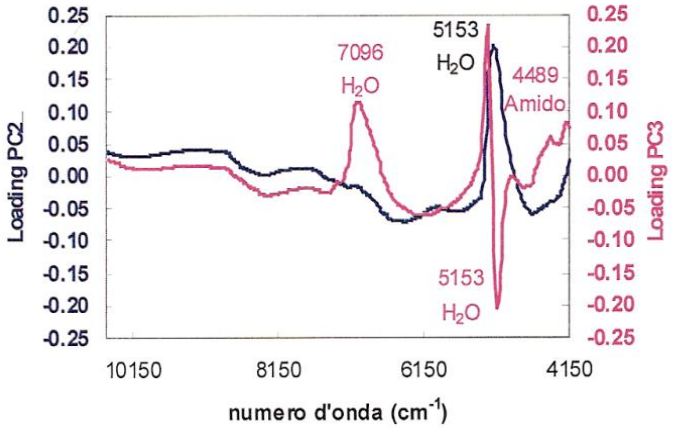


Figura 4.7: PCA Loading Plot del test di lievitazione a 35°C inclusivo delle componenti chimiche coinvolte nella discriminazione

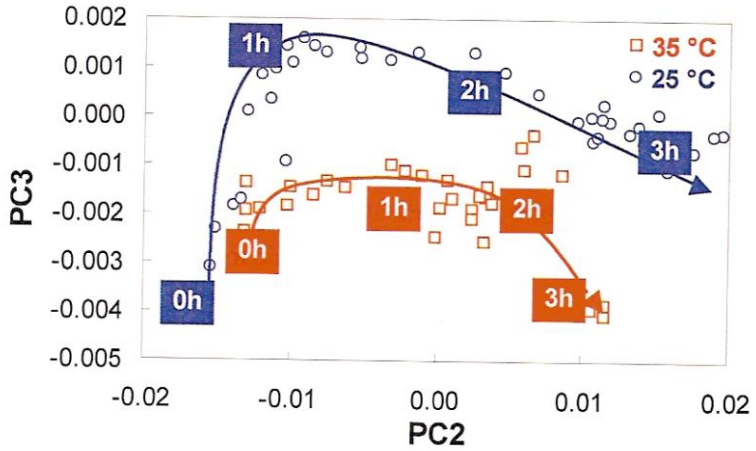


Figura 4.8: PCA Score Plot dei dati spettrali in derivata seconda relativo ai test di lievitazione a 25 e 35°C (le etichette indicano la posizione dei campioni dopo 1,2 e 3 ore di lievitazione)

Second derivative Score e loading plot
Better separation of the series and a clear effect on proteins and gluten appears (C=O)

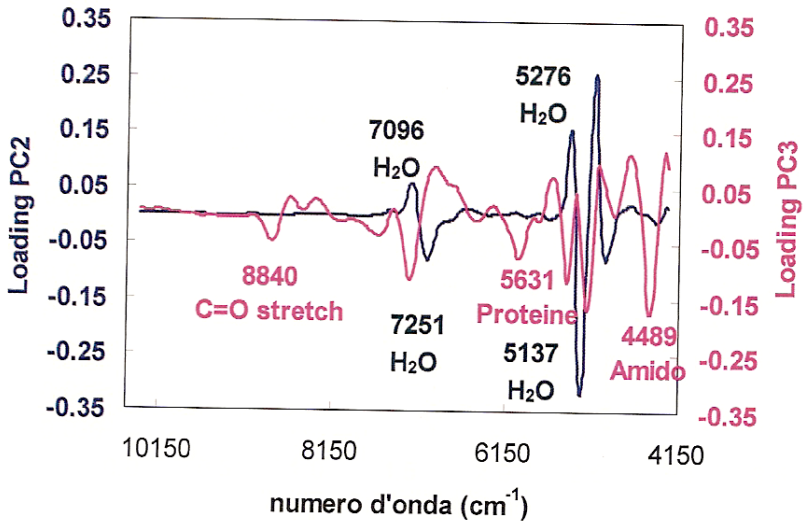


Figura 4.9 : PCA Loading Plot ottenuto dagli spettri in derivata seconda del test di lievitazione a 35°C inclusivo delle componenti chimiche coinvolte nella discriminazione

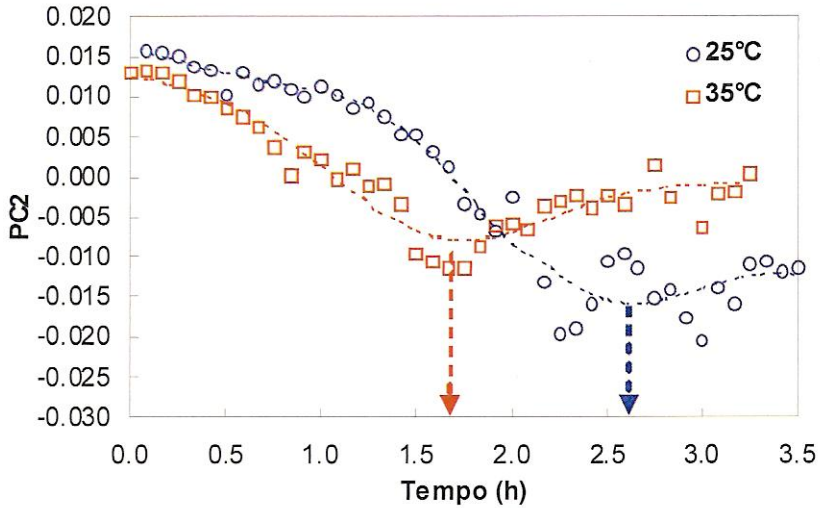


Figura 4.10: Evoluzione dei punteggi della PC2 dei dati spettrali in derivata seconda relativa ai test di lievitazione a 25 e 35°C in funzione del tempo di lievitazione.

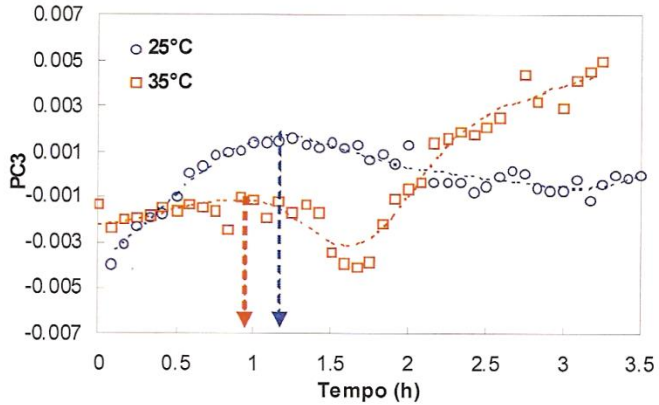


Figura 4.11: Evoluzione dei punteggi della PC3 dei dati spettrali in derivata seconda relativa ai test di lievitazione a 25 e 35°C in funzione del tempo di lievitazione

PC3 gives max speed of the process

Minimun of PC2 corresponding to the collapse of the dough

Temperatura Lievitazione	*Tempo massimo sviluppo (ore)*	*Modello V_t/V_0*			
		Tempo v_max crescita (ore)	*Tempo v_max collasso (ore)*	*Velocità crescita (ore^{-1})*	*Velocità collasso (ore^{-1})*
25 °C	1.55	0.66	2.07	1.15	- 0.24
30 °C	1.16	0.47	1.71	2.04	- 0.16
35 °C	1.07	0.43	1.45	2.28	- 0.40
MIX (40% Avena)	1.19	0.63	1.90	1.82	-0.51

Tabella 4.3: Parametrizzazione delle cinetiche di lievitazione ottenute per Analisi dell'immagine su impasti standard lievitati a differenti temperature

Differences in presence of oats in the dough

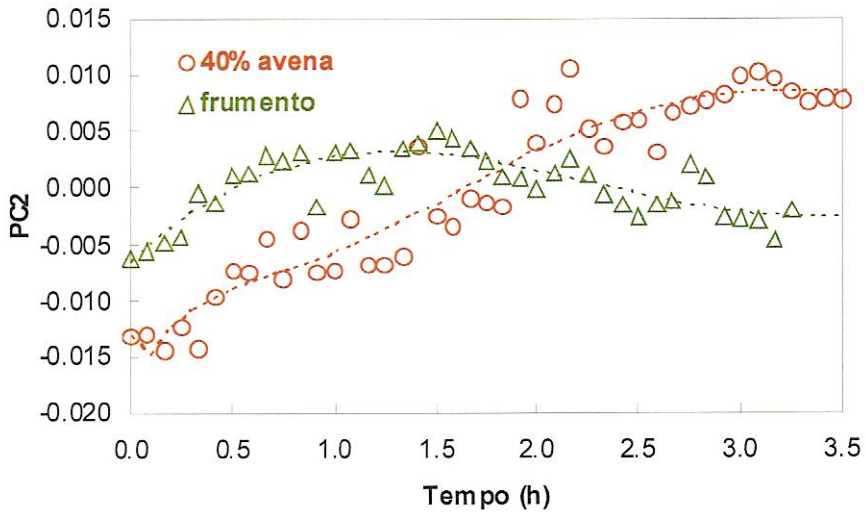


Figura 4.14: Evoluzione dei punteggi della PC2 dei dati spettrali in derivata seconda relativa ai test di lievitazione di miscela di avena in funzione del tempo di lievitazione

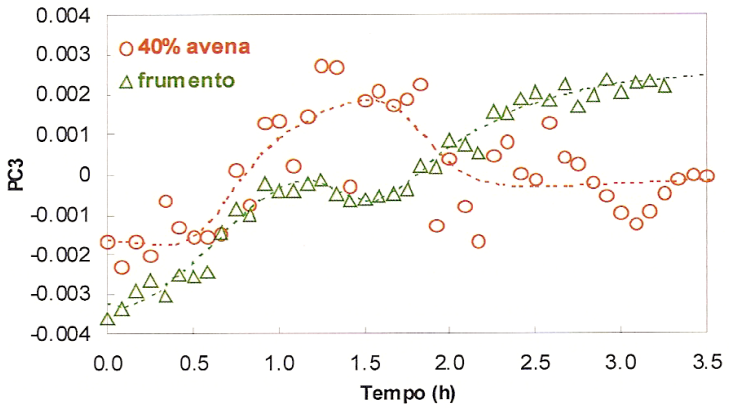


Figura 4.15: Evoluzione dei punteggi della PC3 dei dati spettrali in derivata seconda relativa ai test di lievitazione di miscela di avena in funzione del tempo di lievitazione

Quantitative analysis of :

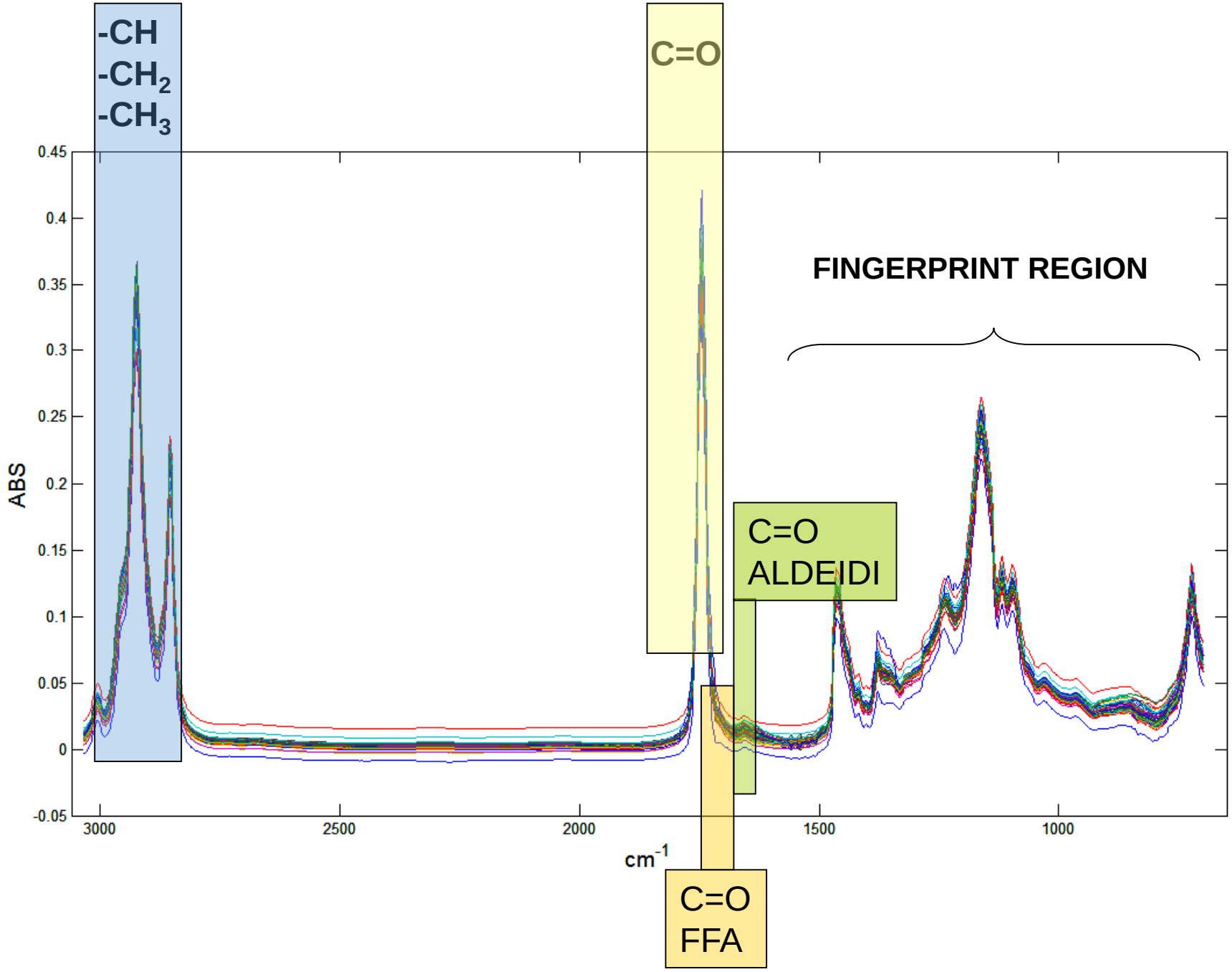
- Oleic acid
- linoleic acid
- Saturated Fatty acids (SFA)
- Monounsaturated fatty acids (MUFA)
- Polyunsaturated fatty acids (PUFA)
- Peroxides value

86 samples of extravirgin olive oil from Abruzzo, Marche e Puglia (2006 e 2007)

- Chemical analysis (FA, PV, spectrophotometric indices)
- Determinaton of fatty acids (GC)

FTIR spectra acquired with Tensor 27™ FTIR (Bruker Optics, Milan, Italy), inteferometer Rocksolid™ and detector DigiTect™ with ATR. ATR (Specac Inc., Woodstock, GA, USA) had ZnSe crystal.

Spectra (32 scans/sample) acquired in the 600 to 4000 cm^{-1} range with a resolution of 4 cm^{-1}.

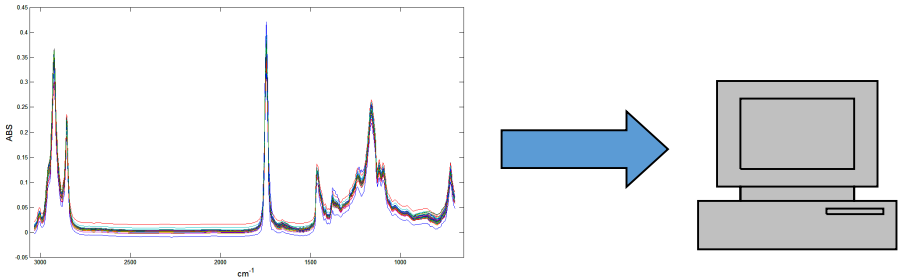

-CH
-CH2
-CH3
C=O
FINGERPRINT REGION
C=O
ALDEIDI
C=O
FFA
ABS
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
3000
2500
2000
1500
1000
cm-1

Data processing and calibration models

Data exported as ASCII file with OPUS 6.0 software and processed with a PLS routine (Partial least squares) run on Matlab (Mathworks Inc., Natick, MA, USA).

For each parameter a PLS model has been built starting from a training set and taking as true value the data obtained using GC or chemical analysis

Spectra initially processed entirely have been reduced using a "moving-windows" strategy with a Matlab routine.



Property	MUFA	PUFA	SFA
Calibration			
Spectral range (cm^{-1})	700-3033	700-3033	700-3033
Linear range (% in VOO)	64 - 81	13 - 20	6 - 16
Number of factors (LVs)	14	15	13
Number of training samples (*N*)	61	61	61
PRESS[a]	10.59	2.52	6.11
Root mean square deviation (RMSD, %)	0.42	0.20	0.32
Relative error in calibration (REC %)	0.56	2.23	1.95
r^2	0.9883	0.9941	0.9557
Selectivity	0.1734	0.1988	0.1378
Sensitivity (SEN)	0.0009	0.0015	0.0020
Analytical sensitivity, [γ= (SEN/σ_o)]	0.17	2.07	0.32
Minimum concentration difference	6.0	0.48	3.17
Limit of detection (LOD, % in VOO)	3	0.28	1.3
Limit of quantification (LOQ, % in VOO)	10	0.94	4.5
Validation			
Number of validations samples	25	25	25
Recovery rates (%)	100	103	98
Relative error in Prediction, (REP, %)	1	4	6
r^2	0.8884	0.9816	0.7099
y_o	5 ± 5	0.4 ± 0.2	5 ± 1
Slope	0.93 ± 0.07	0.98 ± 0.03	0.7 ± 0.1

Results I: oleic and linoleic acid

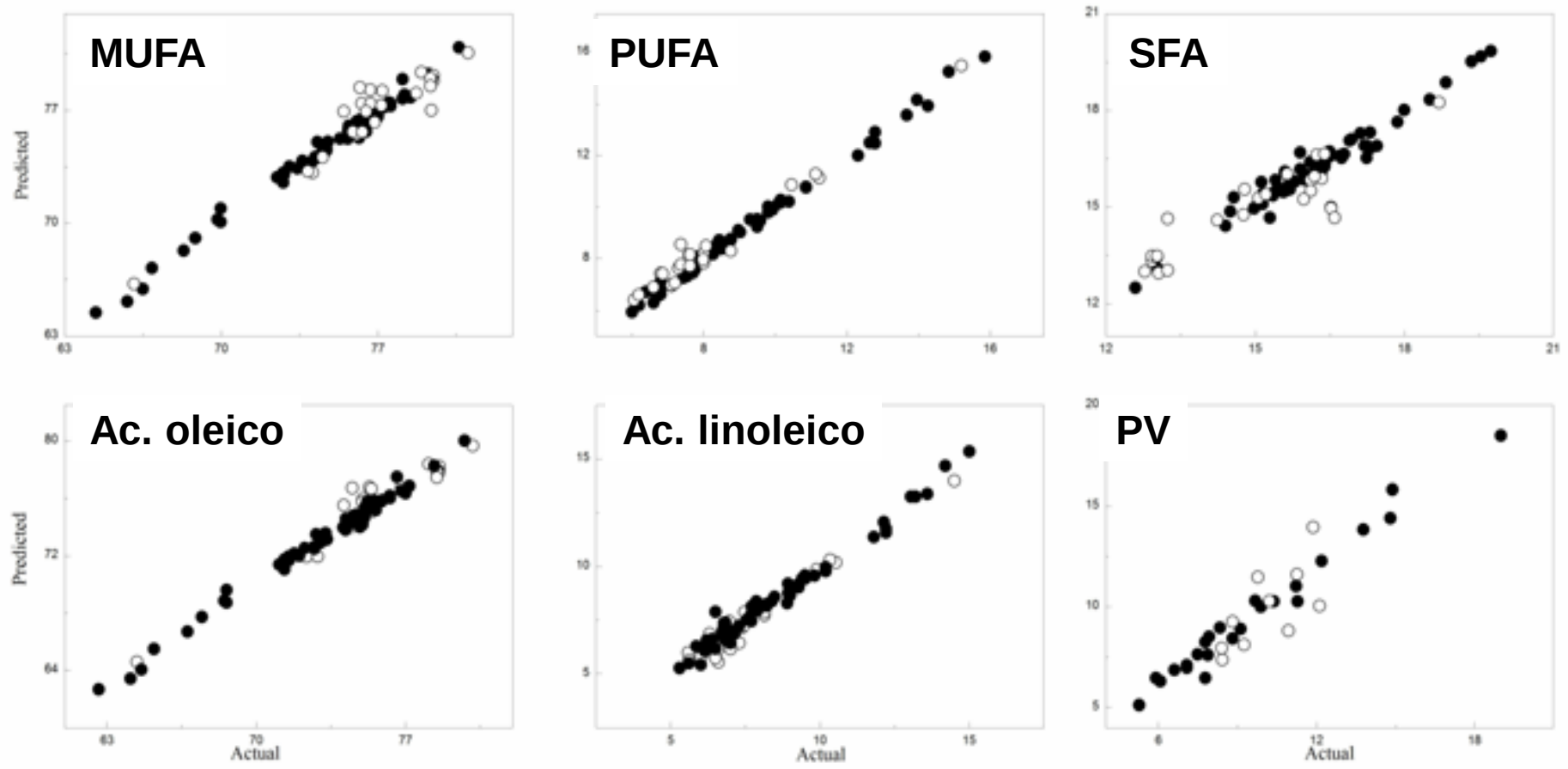
Property	Oleic Acid	Linoleic Acid
Calibration		
Spectral range (cm^{-1})	700-3033	700-3033
Linear range (% in VOO)	62 - 80	5 - 15
Number of factors (LVs)	14	13
Number of training samples (*N*)	61	61
PRESS[a]	10.88	9.33
Root mean square deviation (RMSD)	0.42	0.39
Relative error in calibration (REC %)	0.51	4.64
r^2	0.9886	0.9773
Selectivity	0.1785	0.1988
Sensitivity (SEN)	0.0009	0.0016
Analytical sensitivity, [γ= (SEN/σ_o)]	0.18	1.17
Minimum difference (%)	5.6	0.9
Limit of detection (LOD, % in VOO)	3	0.5
Limit of quantification (LOQ, % in VOO)	10	1.7
Validation		
Number of validation samples	25	25
Recovery rates (%)	100	98
Relative error in Prediction (REP %)	1	7
r^2	0.9232	0.9444
y_o	4 ± 4	0.1 ± 0.4
Slope	0.94 ± 0.06	0.96 ± 0.05

Results II: MUFA, PUFA, SFA

Property	MUFA	PUFA	SFA
Calibration			
Spectral range (cm^{-1})	700-3033	700-3033	700-3033
Linear range (% in VOO)	64 - 81	13 - 20	6 - 16
Number of factors (LVs)	14	15	13
Number of training samples (*N*)	61	61	61
PRESS[a]	10.59	2.52	6.11
Root mean square deviation (RMSD, %)	0.42	0.20	0.32
Relative error in calibration (REC %)	0.56	2.23	1.95
r^2	0.9883	0.9941	0.9557
Selectivity	0.1734	0.1988	0.1378
Sensitivity (SEN)	0.0009	0.0015	0.0020
Analytical sensitivity, [γ= (SEN/σ_o)]	0.17	2.07	0.32
Minimum concentration difference	6.0	0.48	3.17
Limit of detection (LOD, % in VOO)	3	0.28	1.3
Limit of quantification (LOQ, % in VOO)	10	0.94	4.5
Validation			
Number of validations samples	25	25	25
Recovery rates (%)	100	103	98
Relative error in Prediction, (REP, %)	1	4	6
r^2	0.8884	0.9816	0.7099
y_o	5 ± 5	0.4 ± 0.2	5 ± 1
Slope	0.93 ± 0.07	0.98 ± 0.03	0.7 ± 0.1

Results III: PV

Property	D^o	D'	D"
Spectral range (cm^{-1})	4000-700	4000-700	4000-700
Calibration range ($meqO_2\ kg^{-1}oil$)	5.7-15.7	5.7-15.7	5.7-15.7
Number of factors (LV)	5	10	7
Number of training samples	23	24	24
PRESS[a] (unidades)	174.66	152.35	191.32
Root mean square deviation, RMSD (unidades)	1.4302	0.6933	0.9482
Relative error in calibration, REC (%)	15.6	7.2	9.9
r^2	0.8040	0.9759	0.9446
Selectivity	1.0	0.35	0.55
Sensitivity (SEN)	0.0044	0.0001	0.0001
Analytical sensitivity, [γ= (SEN/σ_o)]	1.2	1.1	1.1
Minimum concentration difference (unidades)	0.8	0.9	0.9
Limit of detection (LOD) (unidades)	3.1	1.0	1.6
Limit of quantification (LOQ) (unidades)	10.3	3.4	5.2
Number of validations samples	10	10	10
Recovery rates (%)	74.7	97.7	96.0
Relative error in Prediction, REP (%)	23.7	13.6	12.2



calibration set (●) and trainingset (○)