Application of Transmission FT-IR Spectroscopy for the *trans* Fat Determination in Edible Oils

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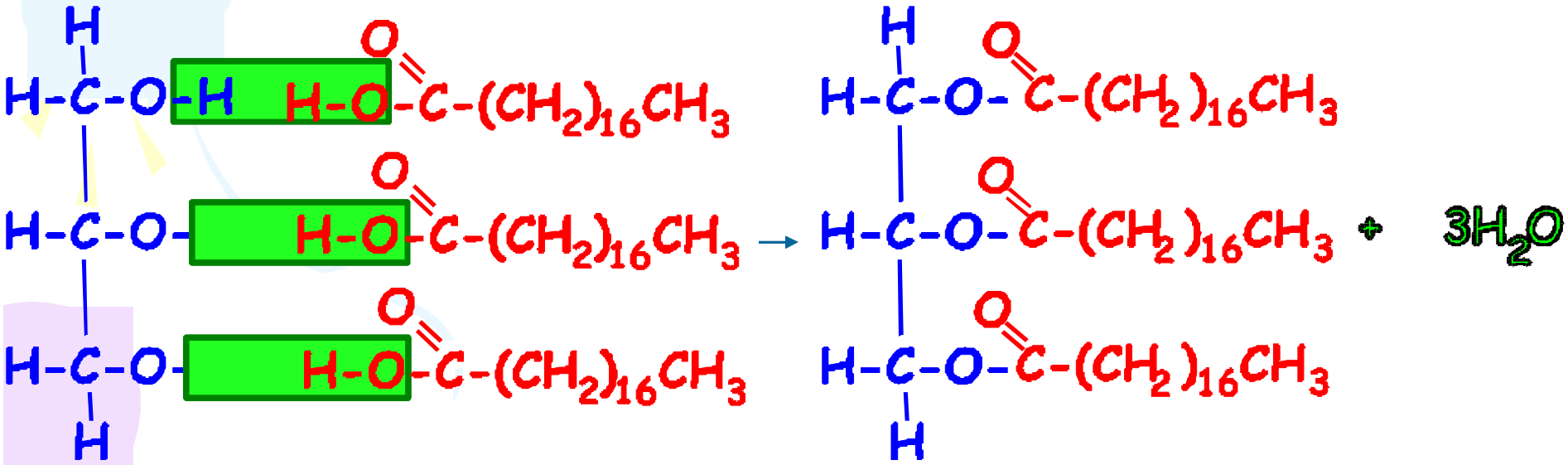
Fats and oils

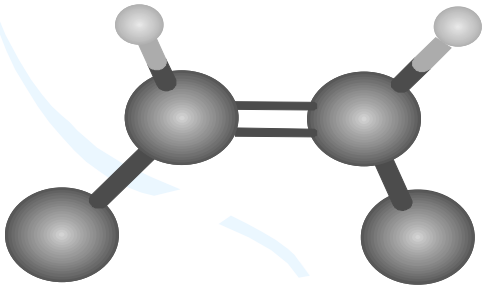
Edible oils are composed of triglycerides, which are the ester of one molecule of glycerol and three molecules of fatty acids



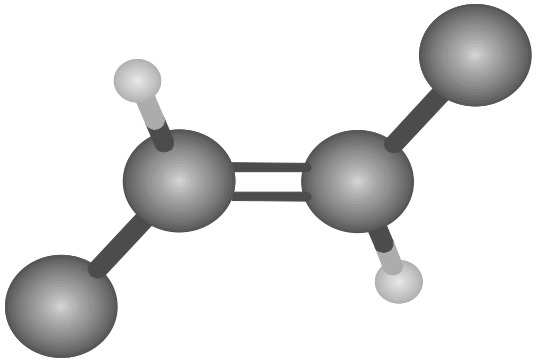
trans Fatty Acids

According to Food and Drug Administration (FDA), *trans* FA are defined as the sum of all unsaturated fatty acids that contain one or more isolated (i.e. non-conjugated) double bonds in a *trans* configuration.

(*trans* FA present in foods / dietary supplements, mandatory for nutritional label since January 2006)



Cis-Unsaturated Chain



Trans-Unsaturated Chain

Health risks of *trans FA*

- **Numerous research and clinical studies determine the impact of *trans* fatty acids on cholesterol levels and coronary heart diseases (CHD).** ***(2 % - 23% risk)***

- **A correlation exist between the intake of *trans* isomers and LDL-cholesterol increase and HDL cholesterol decrease.**

- **Some studies also indicated that TFA raise lipoprotein level, an independent inherited factor of coronary heart diseases.**

Analytical Methods

- Gas chromatography
- ^{13}C NMR spectroscopy
- Infrared spectroscopy

At NCEAC

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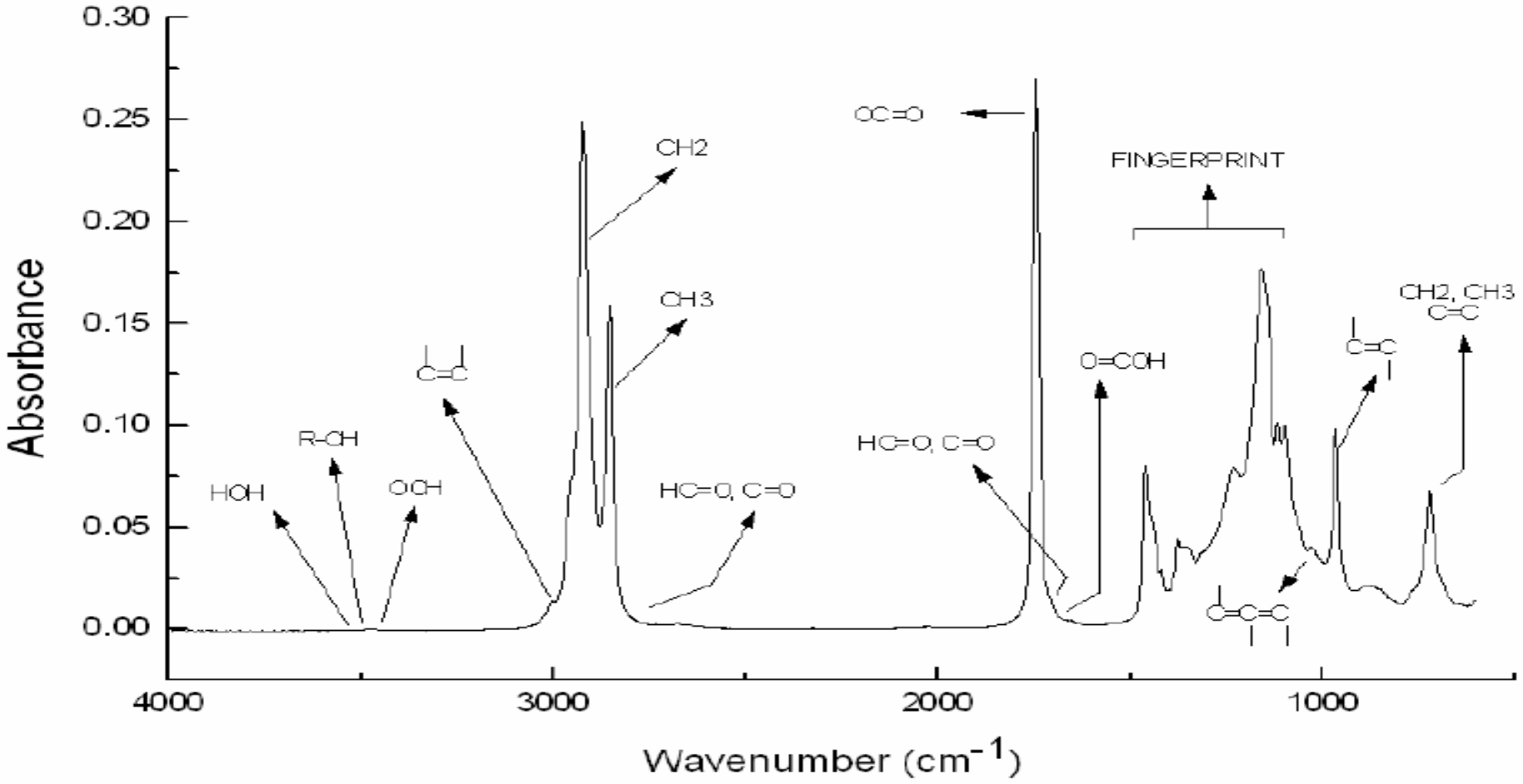
- **Focus on the application of FTIR spectroscopy to food and biological matrices.**
- **Development of rapid instrumental methods for the quantitative analysis of edible oils (FFA, *t*FA, PV, FAC) by FTIR spectroscopy**
- **The rich functional group information available in the mid-infrared portion of the oil spectrum provide a basis for developing a method.**
- **The challenge has been to take a traditionally qualitative procedure quantitative**

FT-IR spectrum of edible oil



IR QUALITY FACTOR INFORMATION

MEASURE	FUNCTIONAL GROUP(S)	CHEMICAL METHOD
DEGREE OF UNSATURATION	C=C	IODINE VALUE
TYPE OF UNSATURATION	*cis*-C=C, *trans*-C=C	TRANS ANALYSIS FATTY ACID PROFILE (GC)
CHAIN LENGTH	CH	SAPONIFICATION NUMBER
HYDROPEROXIDES	OOH	PEROXIDE VALUE
CARBONYL COMPOUNDS	HC=O	ANISIDINE VALUE
CARBOXYLIC ACIDS	COOH	FREE FATTY ACIDS
HYDROXYL GROUPS (MONO/DIGLYCERIDES)	OH	HYDROXYL NUMBER
MOISTURE	OH	MOISTURE CONTENT
SOLIDS CONTENT	CH, C=C, O-C=O	SOLID FAT INDEX

FT-IR spectrometers

Many advantages over traditional dispersive instruments :

- **complete scan in seconds.**
- **multiple scans provide high S/N ratio by signal averaging.**
- **Thus recent advances have made possible to determine not only *trans* FA but also its FA composition without any chemical treatment.**
- **Result – FT-IR spectroscopy now more amenable to development as a quantitative tool.**

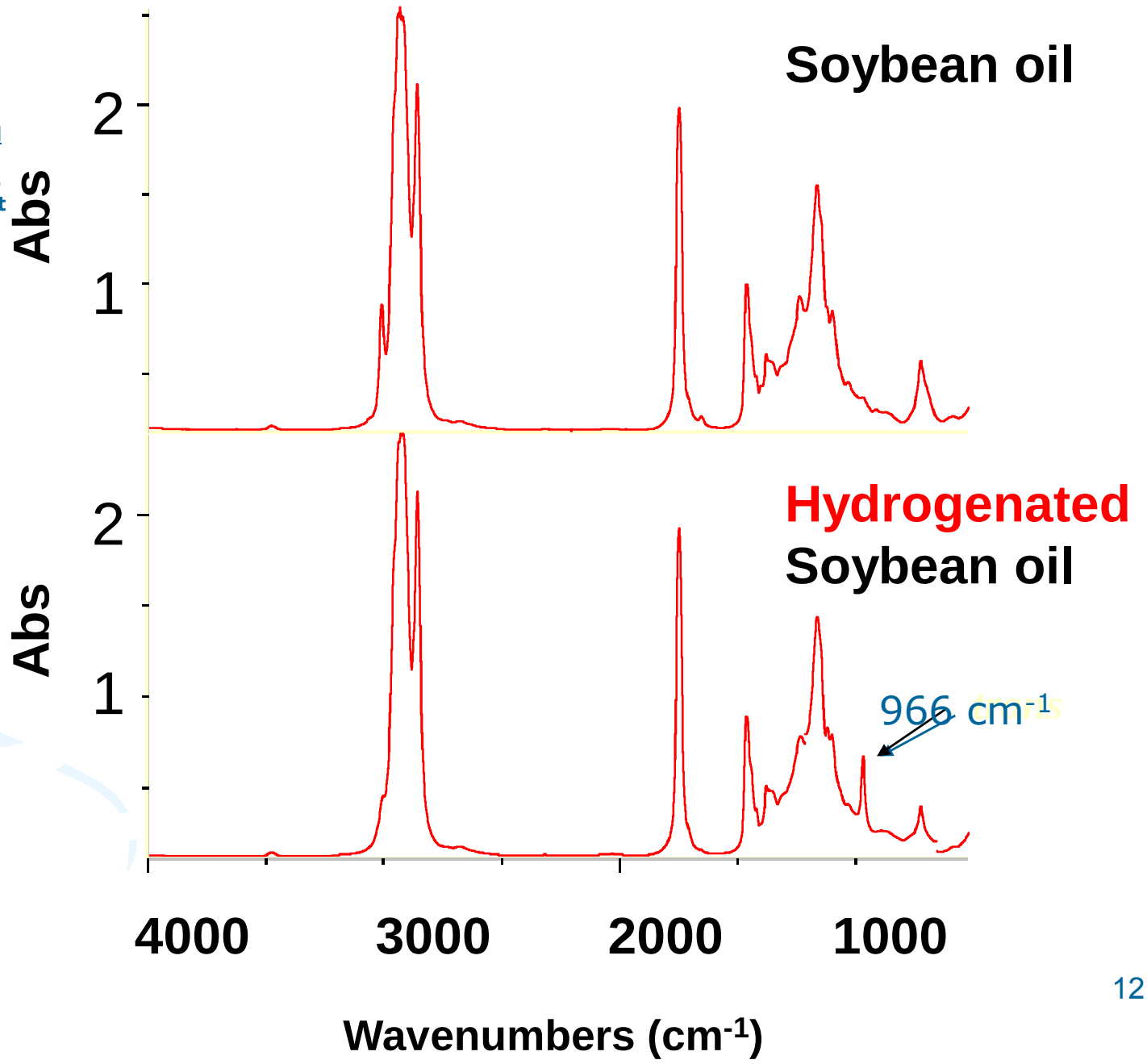
Determination of Isolated *trans* Isomers by Infrared (IR) Spectroscopy

- Method adopted by the American Oil Chemists' Society (AOCS) and AOAC.
- Based on the CH out-of-plane deformation band at 966 cm-1 – a unique characteristic of isolated *trans* isolated double bond.
- Widely employed method, particularly in the analysis of hydrogenated oils in industries.

Factors Limiting Accuracy of Traditional AOCS Method

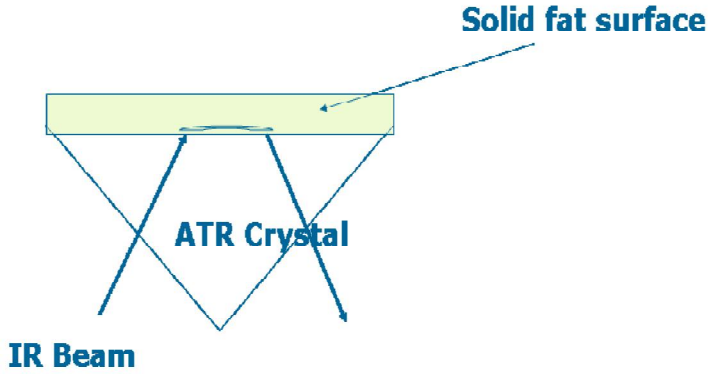
- All triglycerides exhibit a weak absorption band that underlie the ***trans*** absorption band
- Intensity of these underlying absorptions varies with triglyceride composition of the oil; can contribute 3-5 percent to the measured ***trans*** values
- Baseline variability due to intense triacylglycerol absorptions in proximity to the isolated ***trans*** band near 1000 cm^{-1}

In IR spectroscopy the high characteristic *trans* absorption at 966 cm occurs on an elevated and sloping baseline due to acylTG as seen here. Thus measurement of its height and area becomes less accurate especially when trans level goes < 2 %.



Recent modes of FTIR spectroscopy

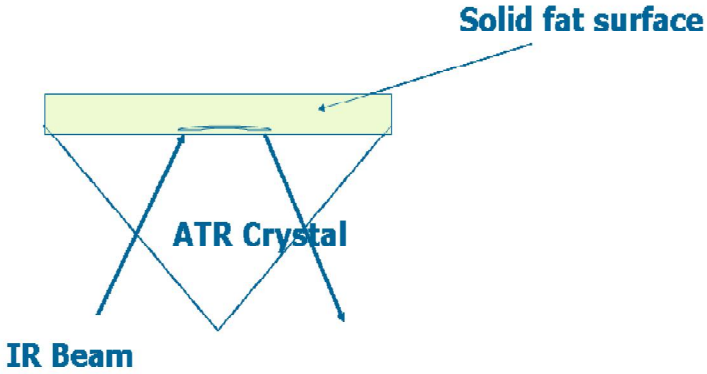
- **Internal reflection or ATR**



- **Transmission FTIR**
- **2 D procedures**

Internal reflection (ATR) infrared spectroscopy

Limitations



Sensitivity

Less sensitivity for trace level tFA due to short path length (~ 4 um)

Accuracy

Transmission FTIR – *an accurate quantitative method for the low level of determination of trans FA.*

- **Fats and oils : High viscosity**

➢ **The limitation of sensitivity / accuracy was overcome by :**

- **Transmission cell - 200 um path-length**
- **Diluting the oil/fat with odorless mineral spirits (OMS) for *trans* content (< 1%).**

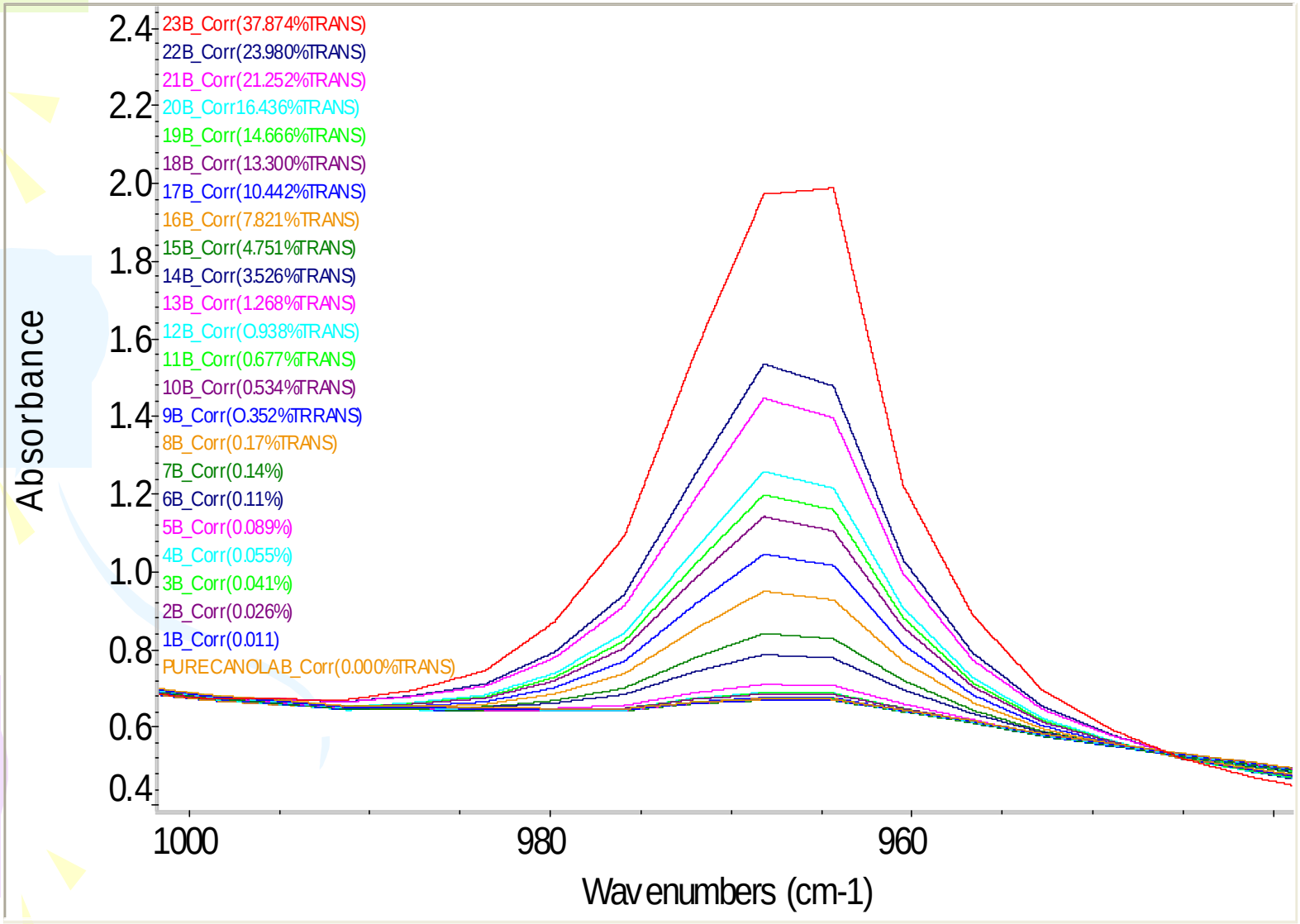
Present Work

In the present study, using transmission FT-IR we have developed a long range of calibration (0.01~37.87%) for the determination of low to high *trans* values in a series of industrially hydrogenated and deodorized oils.

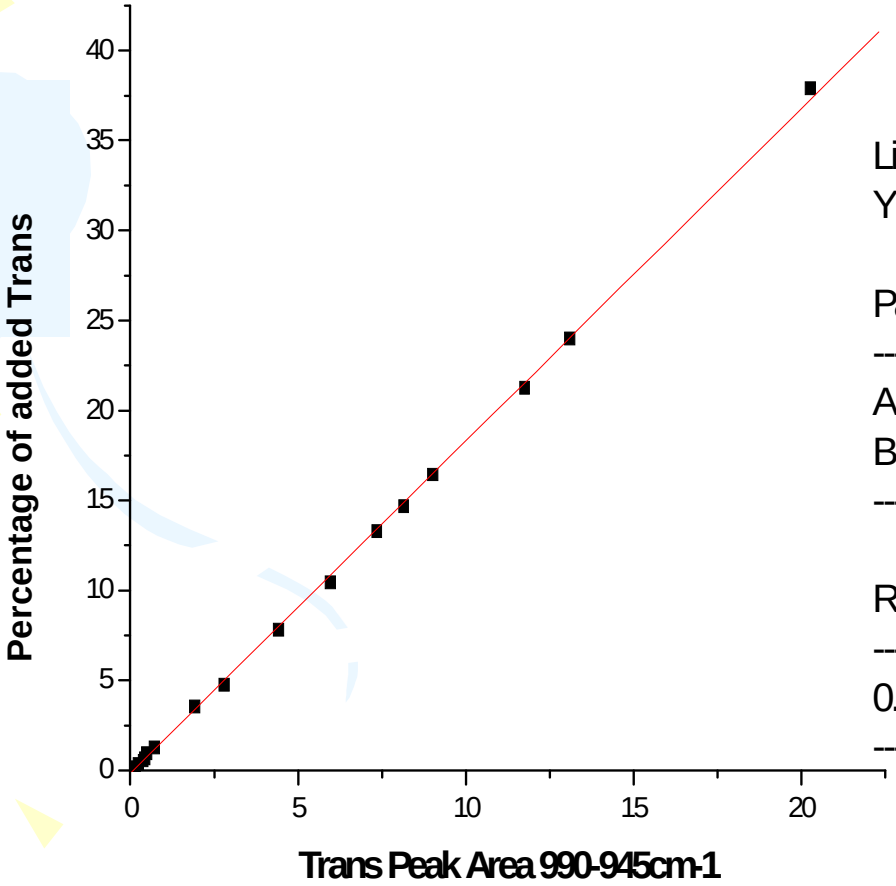
FT-IR Spectral measurements

- Trielaidine ***(t*-elad.)** standards and partially hydrogenated oil are solids at room temperature. The viscosity is reduced by adding OMS in ratio 1:2.
- Standards are prepared by spiking the trielaidine in canola oil for measuring *trans* band at 967 cm^{-1} .
- Prior to the loading of 200 µm KCl cell, all standards and samples were heated to 50 ^{0}C to avoid any crystallization during the analysis.
- The transmission FT-IR spectra of all standards and hydrogenated samples were recorded under the same parameters
- The FT-IR spectra of before deodorization and hydrogenation were subtracted from sample spectra that were recorded after deodorization and hydrogenation process to obtain accurate results.

The absorbance of *trans* band at 967 cm-1 of prepared standards spiking the trielaidine in canola oil ranging from 0.011 to 37.874 %.



Plot of *trans* peak area versus added *trans* to canola oil

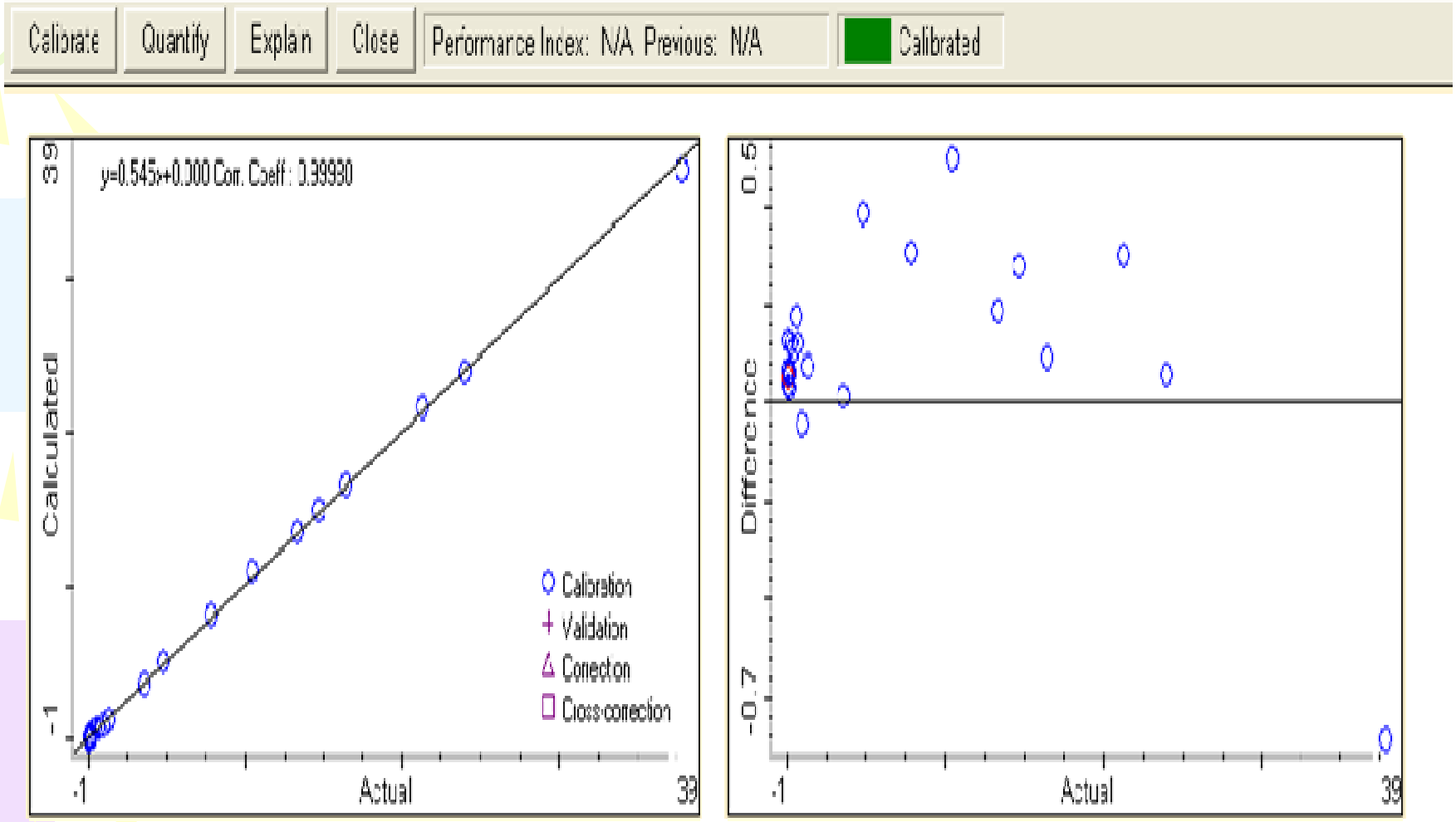


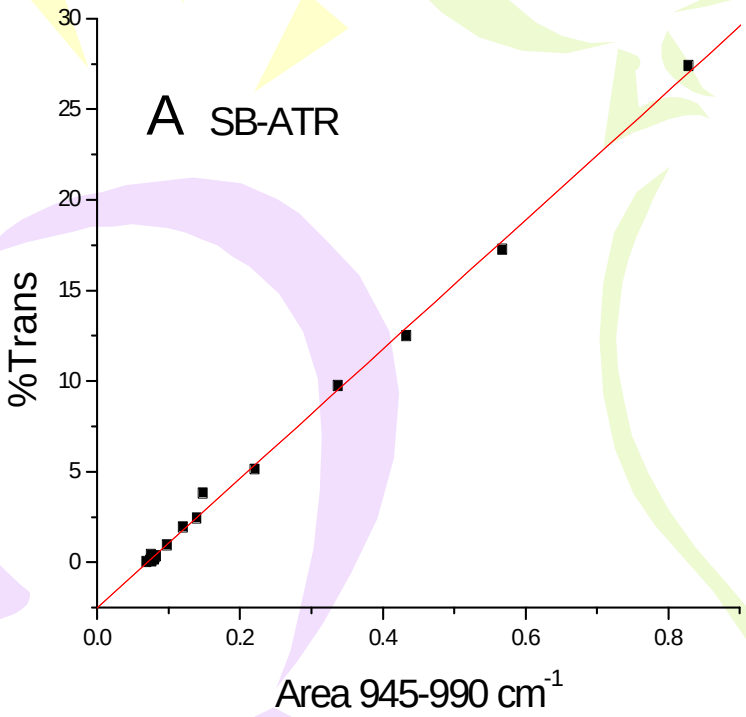
Linear Regression for Data1_Trans:
Y = A + B * X

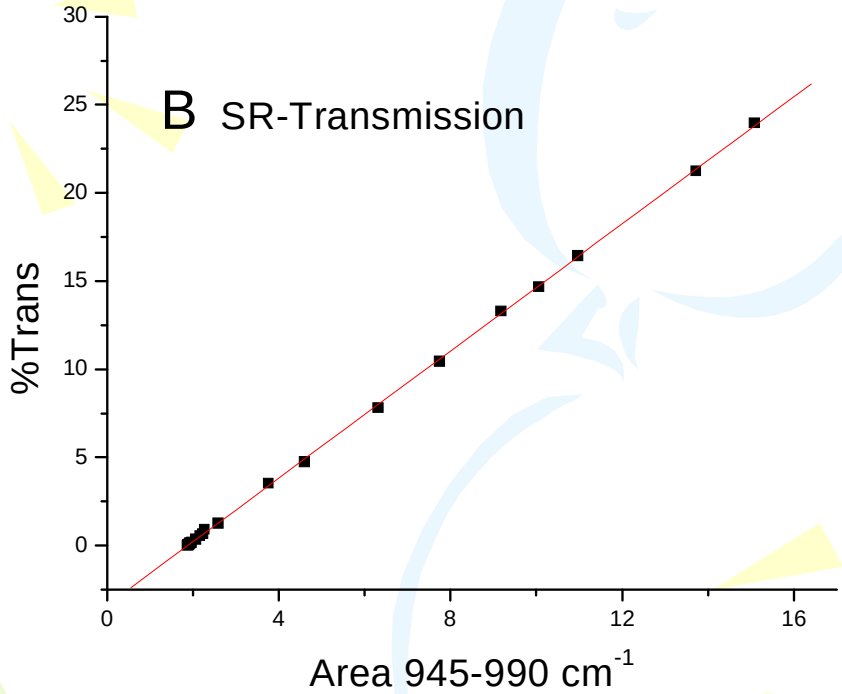
Parameter	Value	Error
A	-0.15776	0.05362
B	1.84724	0.00817

R	SD	N	P
0.99979	0.20926	23	<0.0001

TQ Analyst calibration of the *trans* standards (trielaidine added in canola oil)



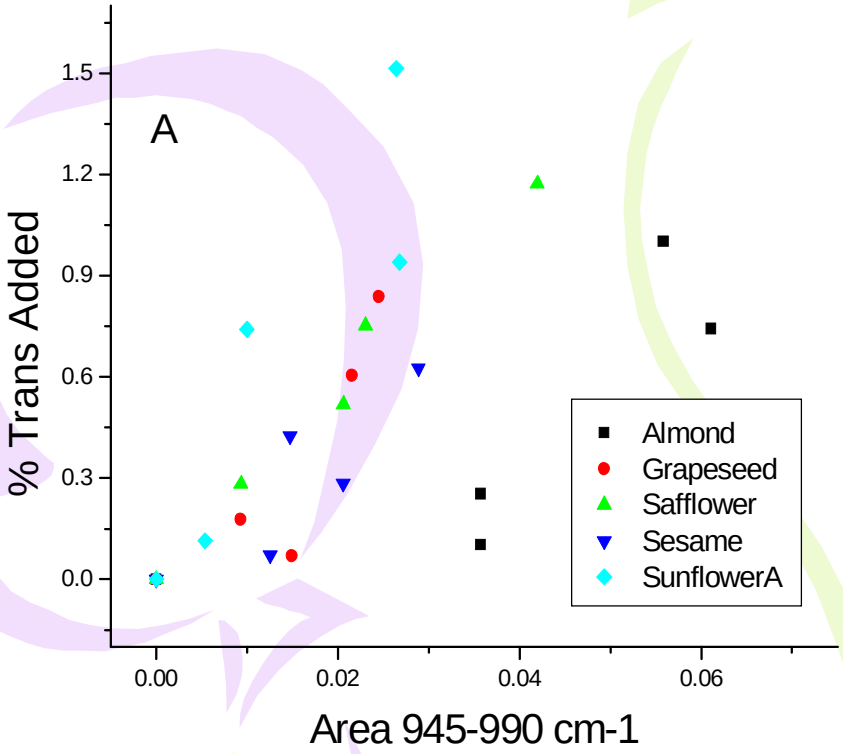




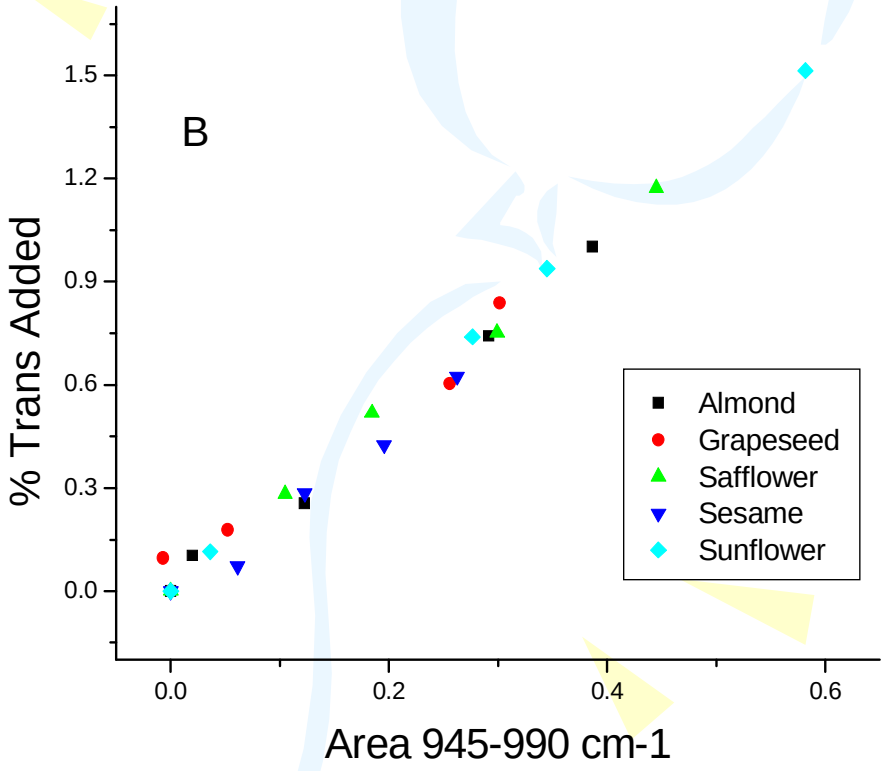
Comparison of FTIR responses of two techniques to increasing level of *trans* hydrogenated oil added to pure canola oil

Sherazi et al. Talanta 80, 600-606, 2009

SB- ATR



T - SR



SB-ATR (A) and T-SR (B) calibration plots obtained from the spectra of five different oils spiked with trielaidin (0 to ~1.2% w/w) ratioed against the spectrum of the unspiked oil. (lower regression SD, more accurate det.)

trans fatty acids (%) in partially hydrogenated oils and pure cooking oils by GC and FT-IR

Samples		GC	FT-IR
1.	PHO-1	9.12 ± 0.23	8.06 ± 0.02
2.	PHO-2	26.51 ± 0.55	25.74 ± 0.05
3.	PHO-3	10.72 ± 0.64	9.61 ± 0.03
4.	PHO-4	10.69 ± 0.22	8.58 ± 0.01
5.	PHO-5	16.32 ± 0.44	15.17 ± 0.04
6.	PHO-6	12.55 ± 0.41	12.21 ± 0.04
7.	PHO-7	20.21 ± 0.61	19.19 ± 0.03
8.	CO-8	0.52 ± 0.05	0.60 ± 0.01
9.	CO-9	0.45 ± 0.16	0.51 ± 0.01
10.	CO-10	0.52 ± 0.12	0.46 ± 0.01
11.	CO-11	1.33 ± 0.21	1.76 ± 0.01
12.	CO-12	1.63 ± 0.23	1.83 ± 0.01
13.	CO-13	1.17 ± 0.16	0.83 ± 0.01
14.	CO-14	1.14 ± 0.23	1.65 ± 0.01

Conclusion

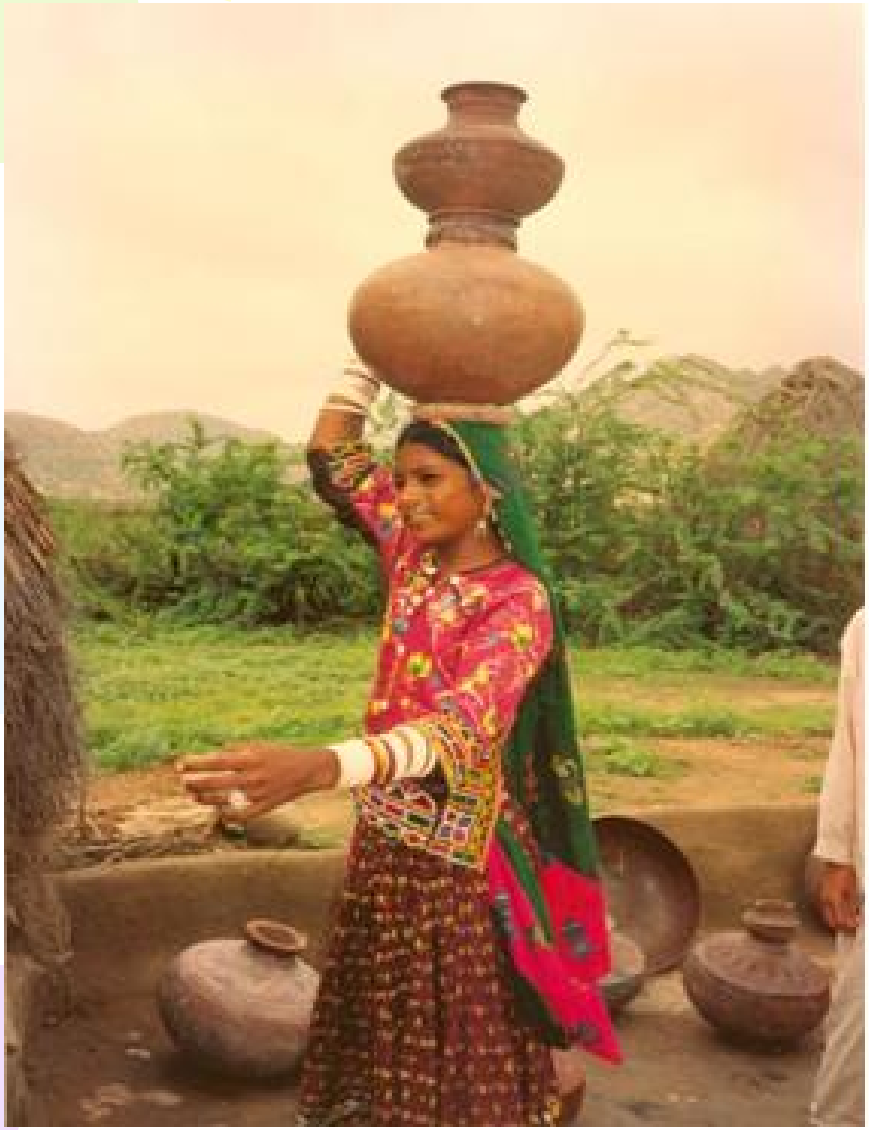
- **FTIR spectroscopy, in conjunction with :**

  developments in sample-handling techniques,
  chemometrics and
  detailed functional-group information provided by mid-IR region

- **is exploited to develop instrumental methods for *trans* Fat in edible oils & fats at very low level.**

- **Accuracy and sensitivity limitations of the FTIR official methods are overcome using transmission FTIR procedure that is currently being validated.**

- **The results of transmission FT-IR spectroscopy were found in good agreement with the GC results and have shown better sensitivity for low *trans (< 1 %)* values in the analyzed edible oil samples.**



THANKS

A young girl of Thar desert in Sindh