RAPID PROPERTIES ANALYSIS OF *PONGAMIA PINNATA* SEED OIL BY NEAR-INFRARED SPECTROSCOPY

The Authors :

Chutarat Khamchum[1], Vittaya Punsuvon[1,2,3], Potjanart Suwanruji[1], Sumaporn Kasemsumran[4] and Nattaporn Suttiwijitpukdee[4]

[1]Department of Chemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand
[2]Center of Excellence-Oil Palm, Kasetsart University, Bangkok 10900, Thailand
[3]Center of Advanced studied in Tropical Natural Resource, NRU-KU, Kasetsart University, Bangkok 10900, Thailand
[4]Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI), Kasetsart University, Bangkok 10900, Thailand

Presented by

Chutarat Khamchum

Department of Chemistry, Faculty of Science,
Kasetsart University

Outline

- **Introduction**
- **Objective**
- **Materials and Methods**
- **Results and Discussion**
  - Fatty acid composition
  - NIRS analysis
  - Calibration model
  - External prediction
- **Conclusion**

Introduction

Pongamia Pinnata

http://wildshores.blogspot.com/2009/04/special-shore-tree-on-changi-pongamia.html

- It is a medium sized glabrous tree belonging to the family *Leguminacene*
- In the south of Thailand, found on Andaman coast such as Ranong, Krabi, Phuket provinces.
- Known as "*Hye Ta Lay*" or "*Hye Nam*"
- It is highly tolerant of salinity and can be propagated either by seeds or root suckers.

Introduction

Pongamia Pinnata

(a)

(b)

Pongamia Pinnata (a): pods and (b): kernels

Introduction

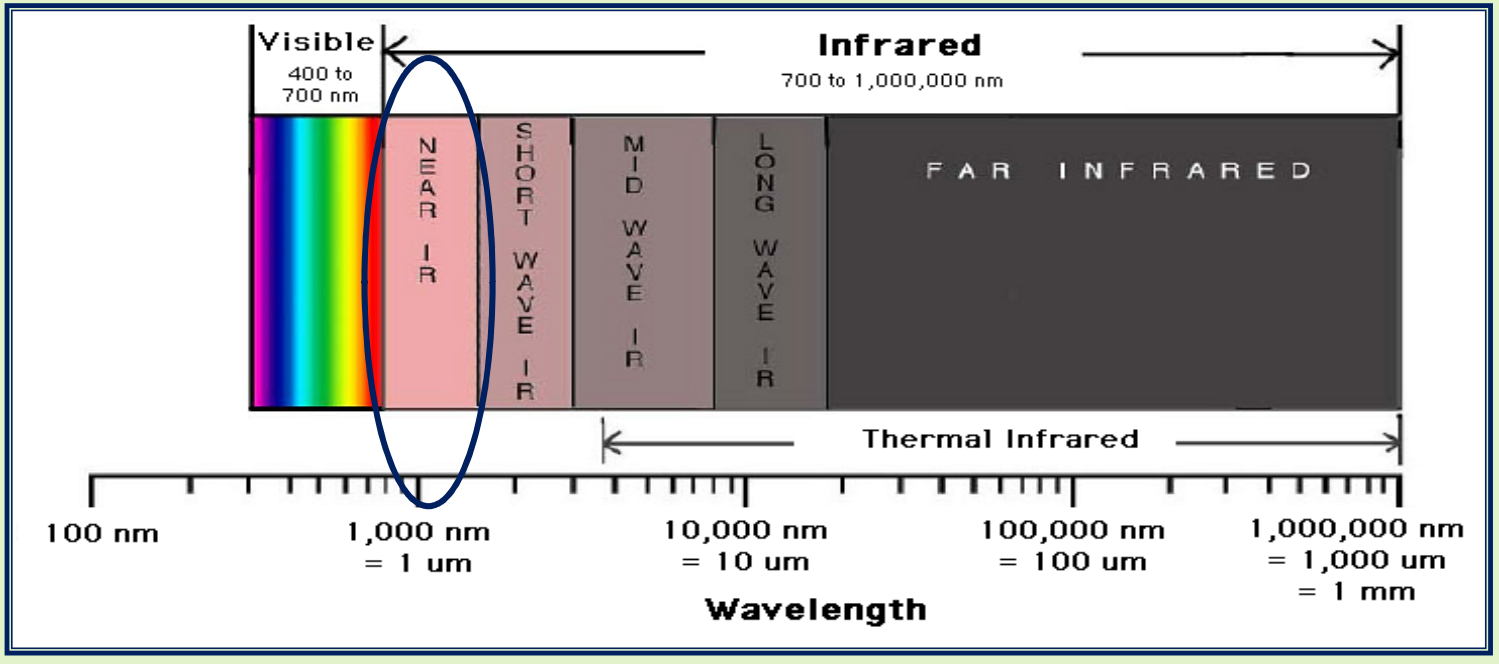
Pongamia Pinnata oil

- The oil content about 17-28%
- The oil contain several toxic compounds, such as karanjin, pongapin and pongaglubin
- The oil is rich in C18:1 fatty acid (oleic acid)
- Has potential to be a raw material for biodiesel production.

Introduction

Near Infrared Spectroscopy (NIR)

- **Absorption of electromagnetic radiation.**
- **Region ⟶ 800-2500 nm (12,820-4,000 cm^{-1})**



http://mvh.sr.unh.edu/mvhtools/near_ir.htm

Introduction

Near Infrared Spectroscopy (NIR)

- NIR is typically used for quantitative measurement of organic functional groups, especially O-H, N-H, and C=O
- Absorption of radiation is used to develop calibration curves, which can be related to sample properties.
- Regression equations developed allows accurate analysis of many other samples by prediction of data based on the spectra.
- Applications include pharmaceutical, agricultural, polymer, and clinical analysis.

Introduction

Near Infrared Spectroscopy (NIR)

How to use NIRS ?

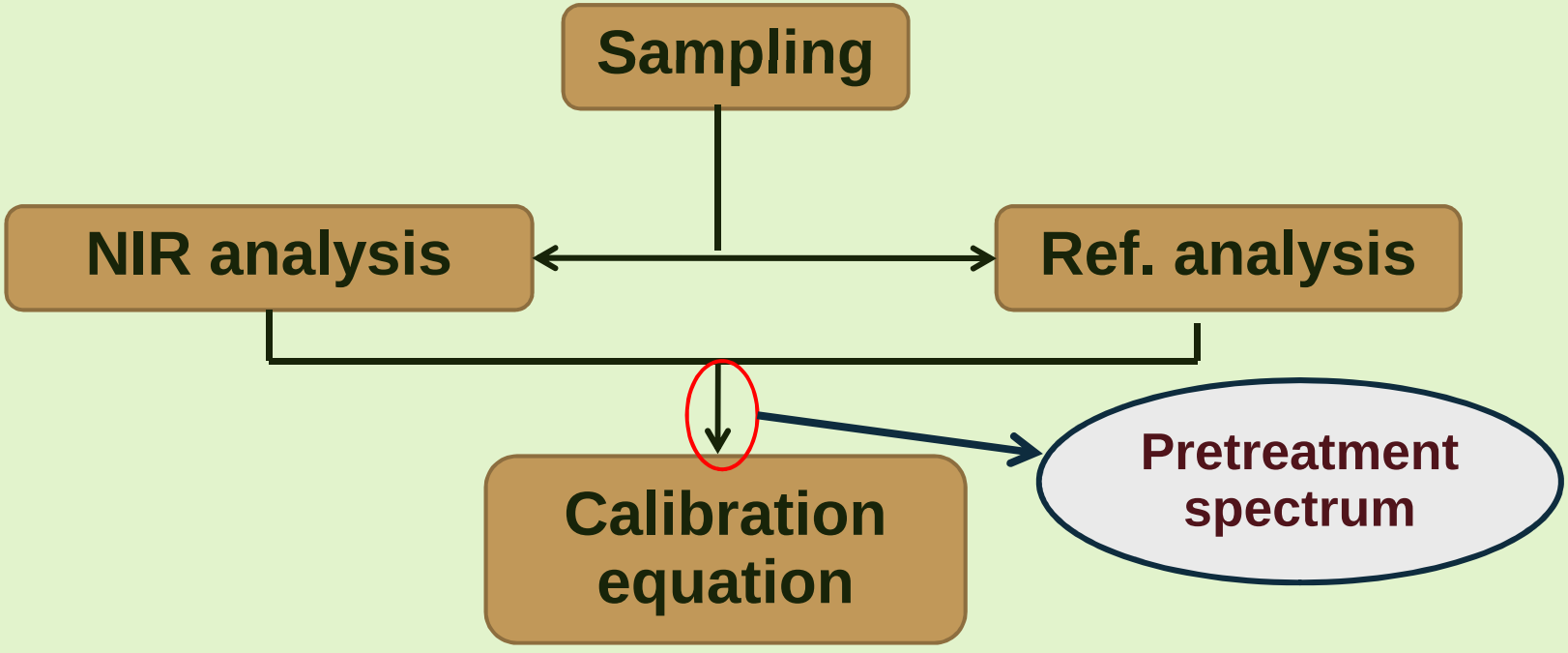
3 Steps of NIRS analysis

- ❑ Calibration equation
- ❑ Validation test
- ❑ Predicted unknown samples

Introduction

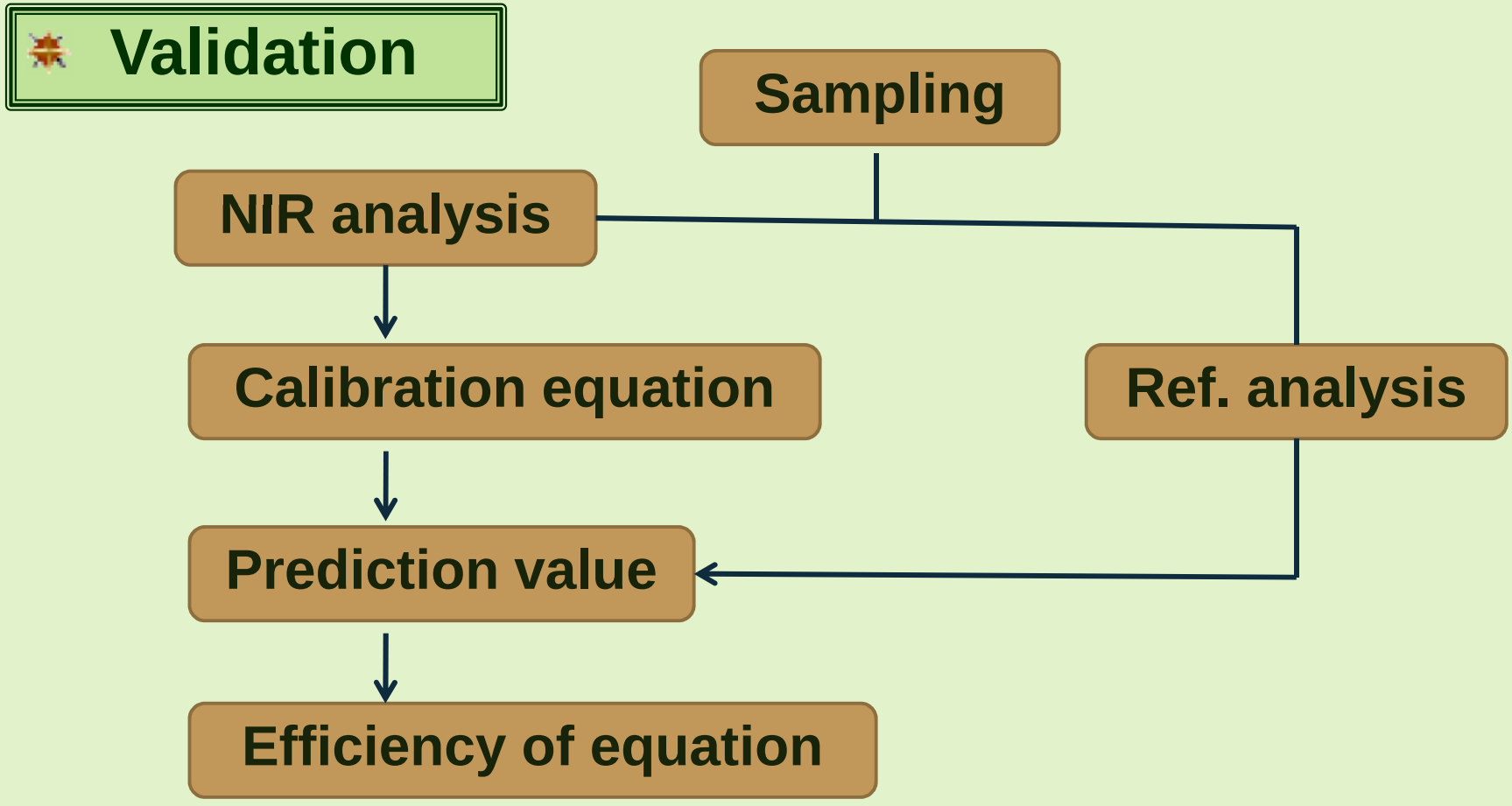
Near Infrared Spectroscopy (NIR) : Steps

- **Calibration model / equation**



Introduction

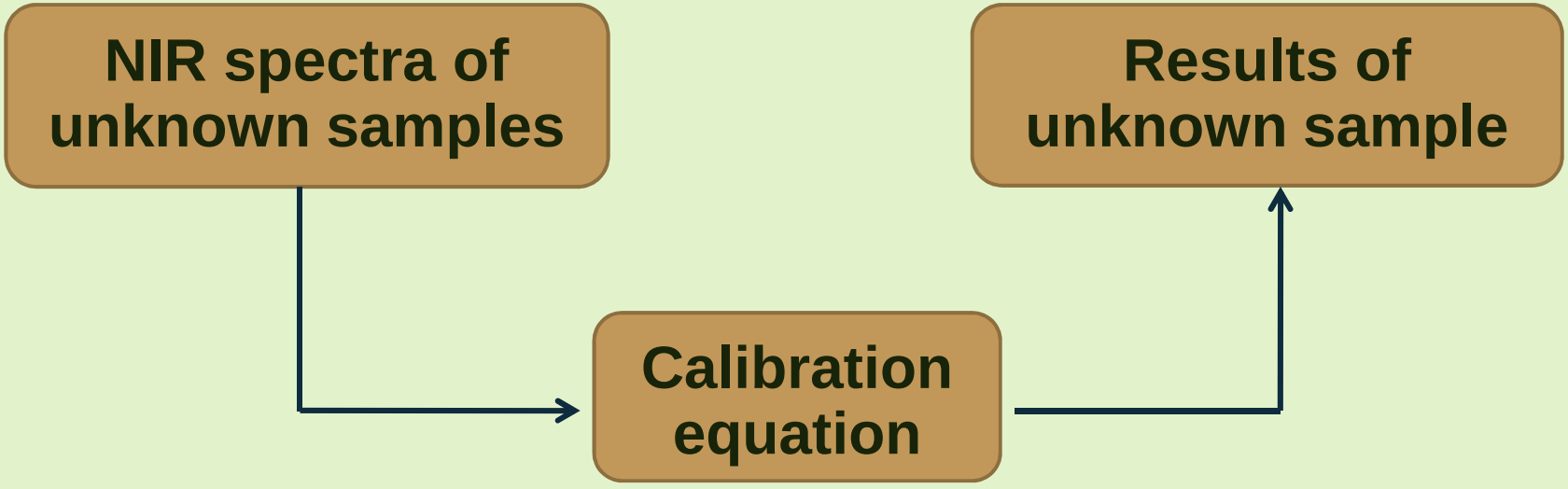
Near Infrared Spectroscopy (NIR) : Steps



Introduction

Near Infrared Spectroscopy (NIR) : Steps

- **Prediction of unknown sample**



Introduction

Near Infrared Spectroscopy (NIR)

Advantages

- Cheaper and faster alternative
- Non-destructive sample
- Requires minimal sample preparation
- Non-chemical, good for environment
- Response in real time

Objective

To investigate the feasibility of predicting

- **Fatty acid composition**
- **Free fatty acid content (FFA)**

in Pongamia Pinnata oil by using Near Infrared Spectroscopy (NIRS)

Materials and Methods

Samples preparation

- 60 sample seeds were collected from the southern part of Thailand
- All seeds were dried under room conditions
- The hull's pods were manually removed
- Kernels were ground by grinder and sizing with sieve (40-60 mesh)

P. Pinnata powder

Materials and Methods

Oil extraction

Powders

Soxhlet Extraction

70 ˚C, 4 hr.

Oils

Free fatty acid content (FFA)
by AOCS Ca 5a-40 method

Fatty acid compositions
by AOAC 969.9 method and analyze by GC

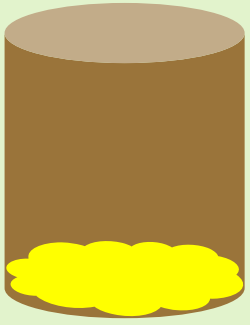
Materials and Methods

Free fatty acid content

Sample oils

+ Ethanol

Phenolpthalein



Solutions

+ 0.25N NaOH

$$\%FFA = \frac{\text{ml of NaOH x conc.NaOH(N) x 28.2}}{\text{weight of samples}}$$

Materials and Methods

Fatty acid composition

Sample oils

+ Methanol

NaOH

90˚C, 30 min

BF_3

90˚C, 2 min

NaCl + Heptane

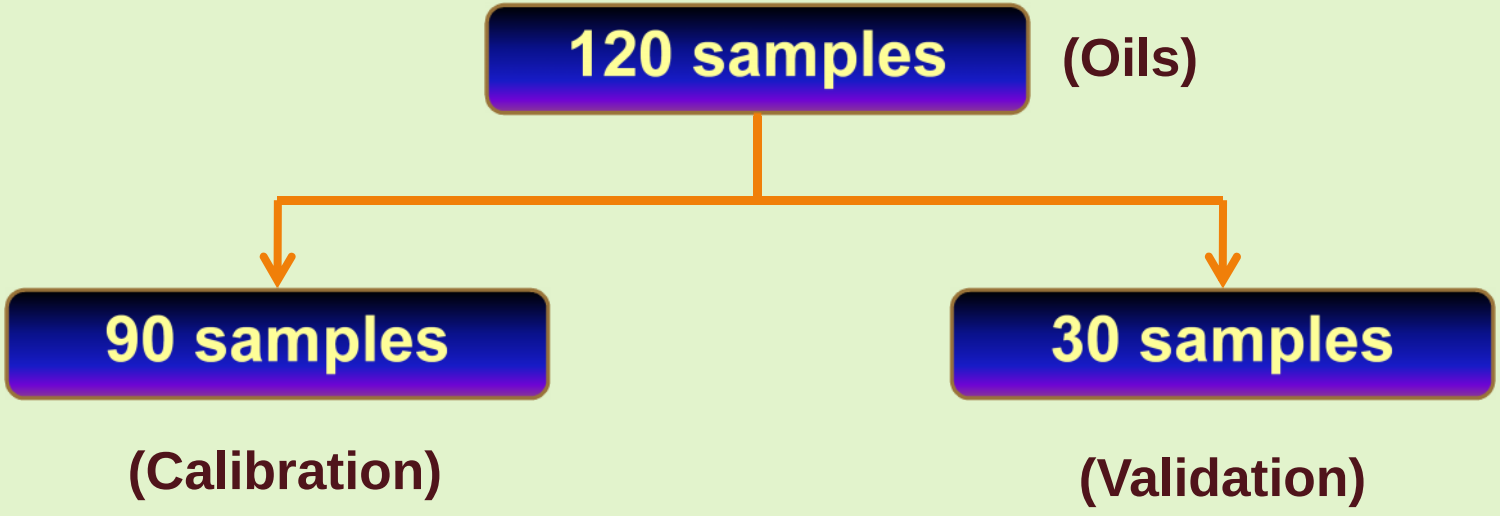
Analyzed by GC

(Solution ‘s separated)

Materials and Methods

NIRS analysis

To estimate free fatty acid content and fatty acid composition



Materials and Methods

NIRS analysis

Sample oils

room temp.

Liquid cell

Drop the sample oils into liquid cell (1-2 ml)

Cover samples with gold piston

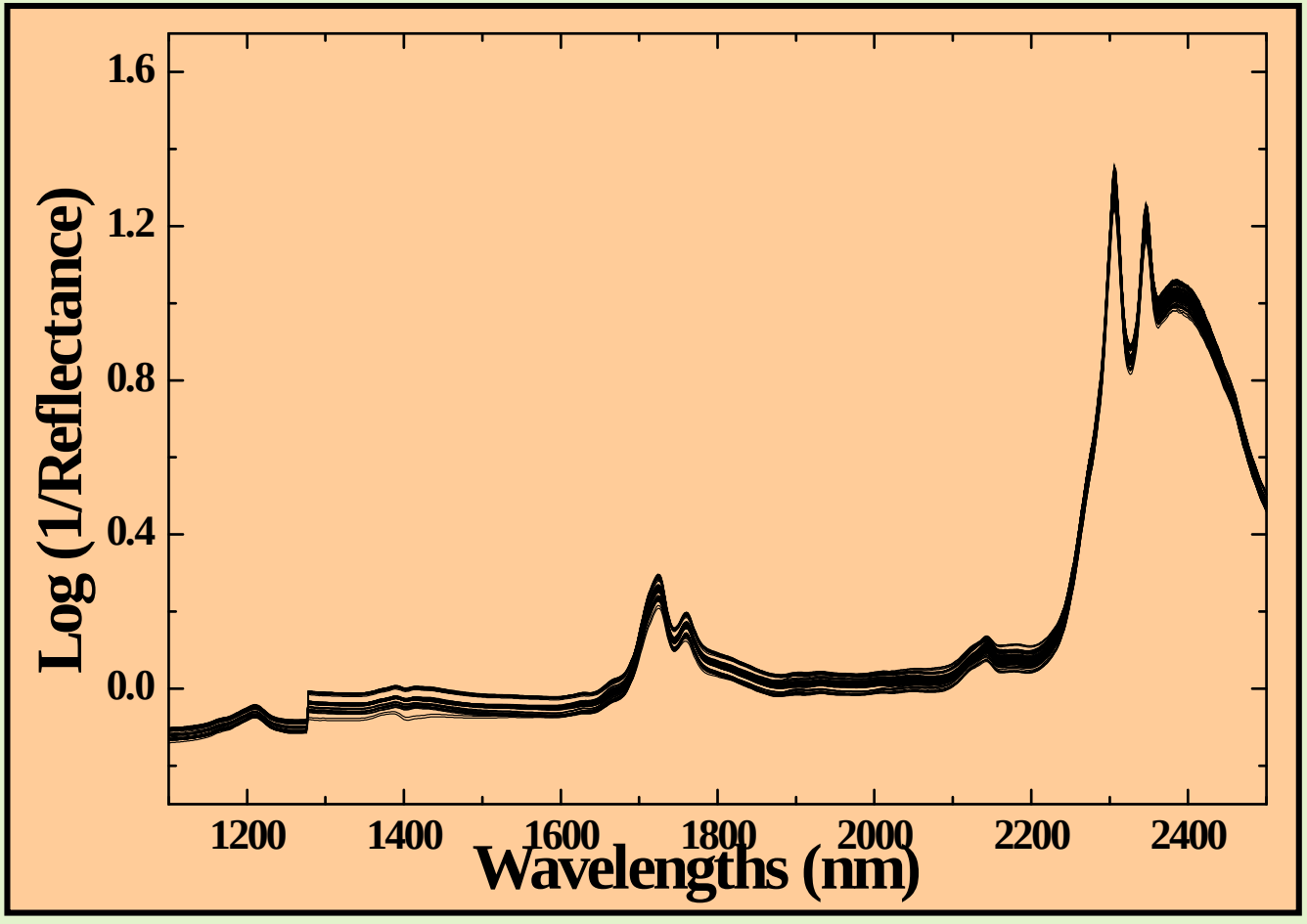
NIR (Unity, Spectrastar)

Range: 1100-2500 nm

Materials and Methods

NIRS analysis

Range: 1100-2500 nm



Spectrums data

Materials and Methods

Spectral pre-treatment and calibration

Chemometrics

Savitzky-Golay first derivative (1De) was combined with other techniques as:

- **Smoothing (SM)**
- **Normalize (NM)**

Calibration equations

By Partial least squares (PLS) regression

Statistical analyses were performed with the Unscrambler 9.8 software (Camo).

Materials and Methods

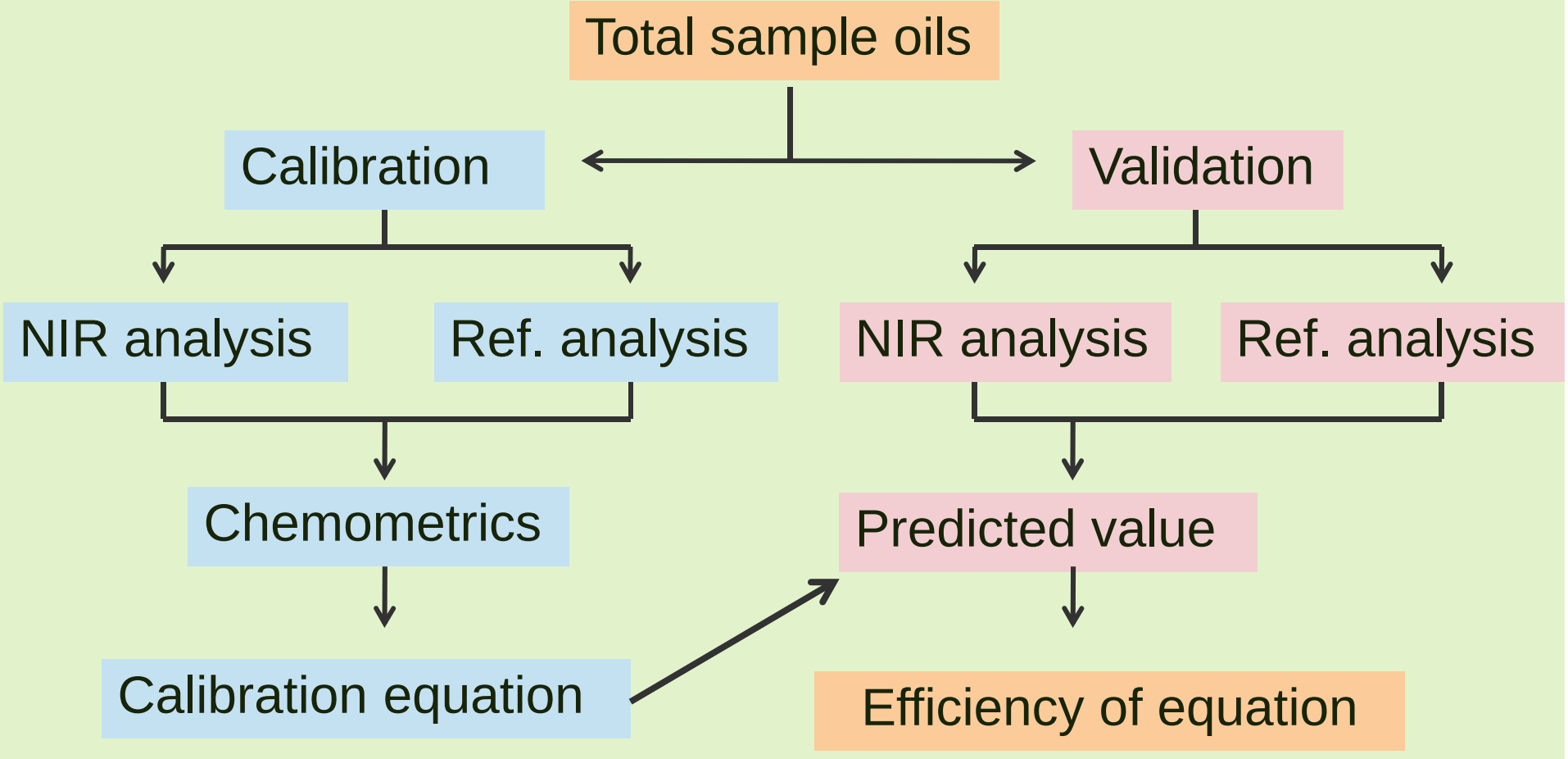
Spectral pre-treatment and calibration

The indicators of prediction quality

- The correlation coefficient of determination (R) ↑ > 0.7
- The standard errors of calibration (SECV) ↓
- The standard errors of prediction (SEP) ↓
- Bias ↓
- Relative predictive determination (RPD) ↑ > 1.5

Materials and Methods

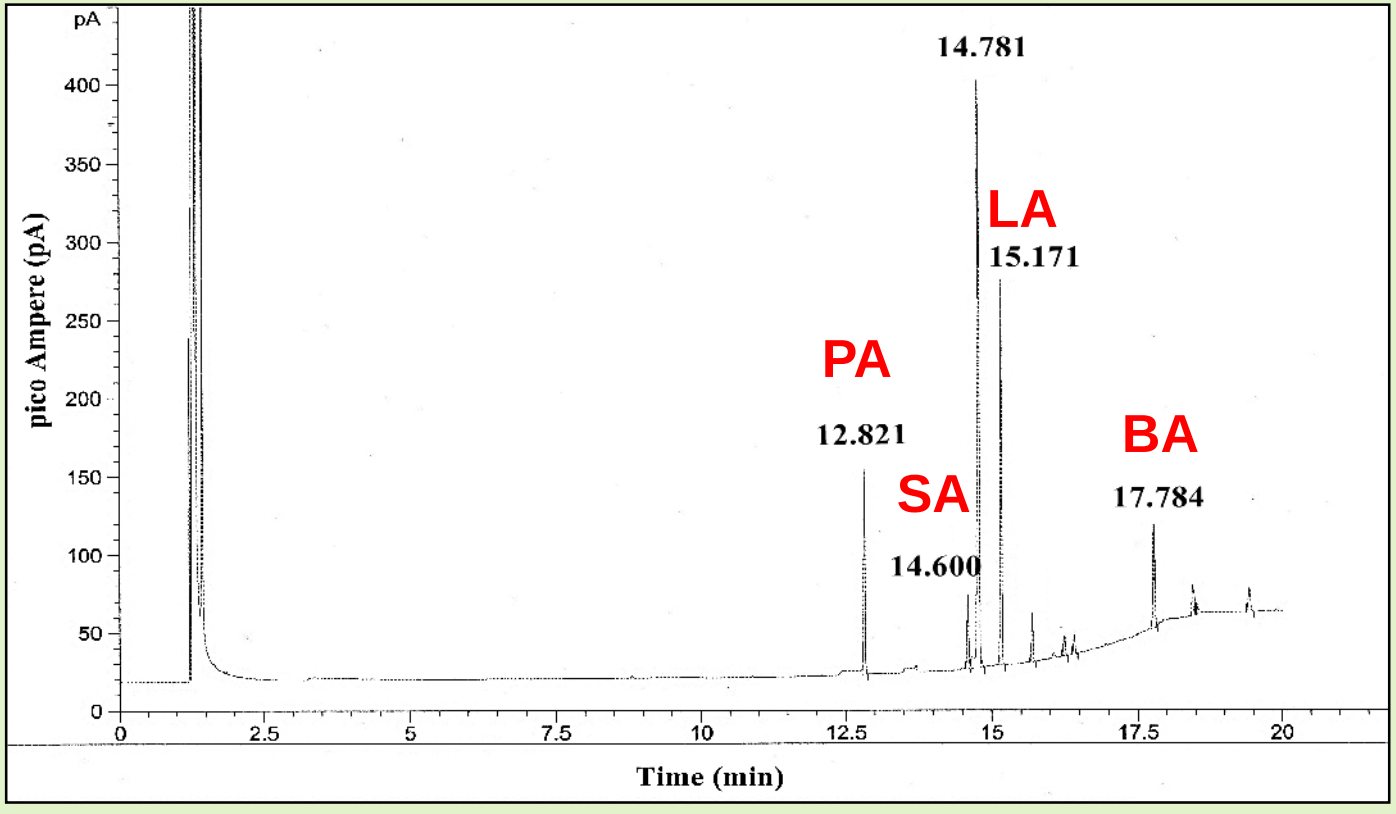
Summary of all steps for NIR analysis



Results and Discussion

Fatty acid composition

35 - 50 %



Chromatogram

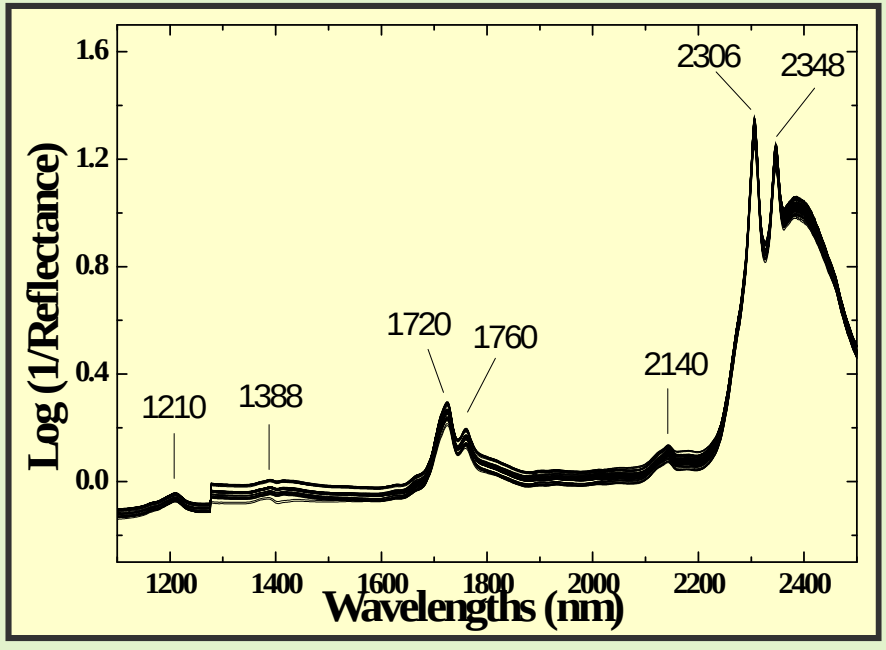
Results and Discussion

Fatty acid composition and free fatty acid content

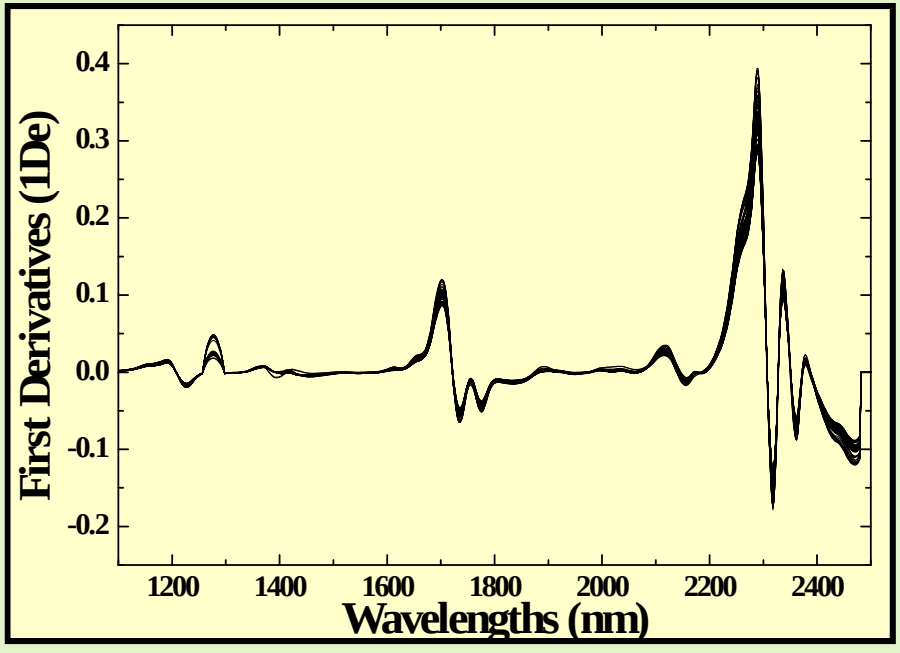
Composition	Content (%)
Palmitic acid (C16:0)	10.36 – 15.69
Stearic acid (C18:0)	2.66 – 13.84
Oleic acid (C18:1)	35.01 – 50.65
Linoleic acid (C18:2)	10.03 – 26.87
Behenic acid (C22:0)	4.15 – 12.87
FFA	1.03 – 35.40

Results and Discussion

NIRS analysis



The original NIRS spectra



After pretreat with 1st derivatives (1De)

Results and Discussion

Calibration model

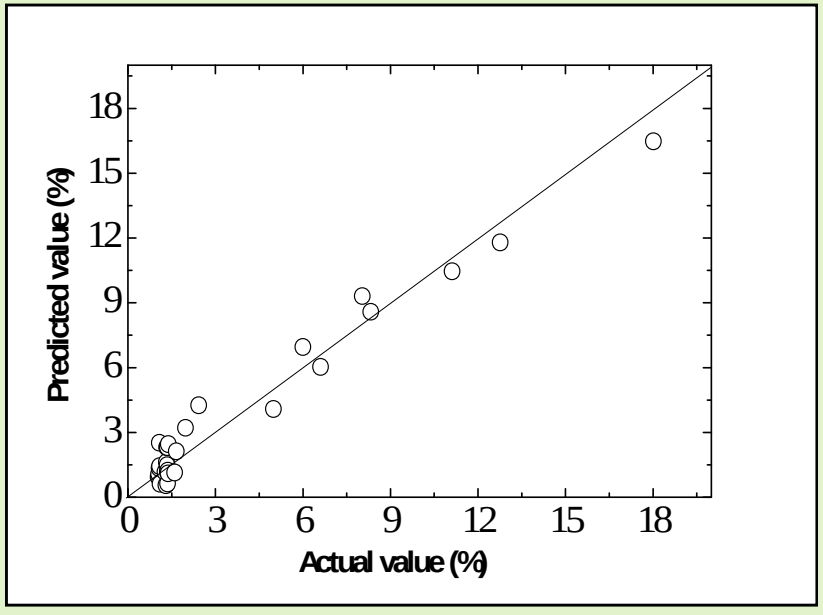
Summary pretreatment techniques in **“calibration model”**

Contents	Pretreatment	F	Calibration			
			R	SECV	Bias	
FFA	SM + 1De	4	0.977	0.873	-0.022	good
C18:1	SM	12	0.859	1.893	0.033	
C18:2	NM + 1De	12	0.792	2.461	-0.026	
C16:0	NM + 1De	10	0.693	0.928	-0.004	poor
C18:0	NM + 1De	13	0.841	1.019	0.000	
C22:0	SM + 1De	12	0.774	1.169	-0.023	

Results and Discussion

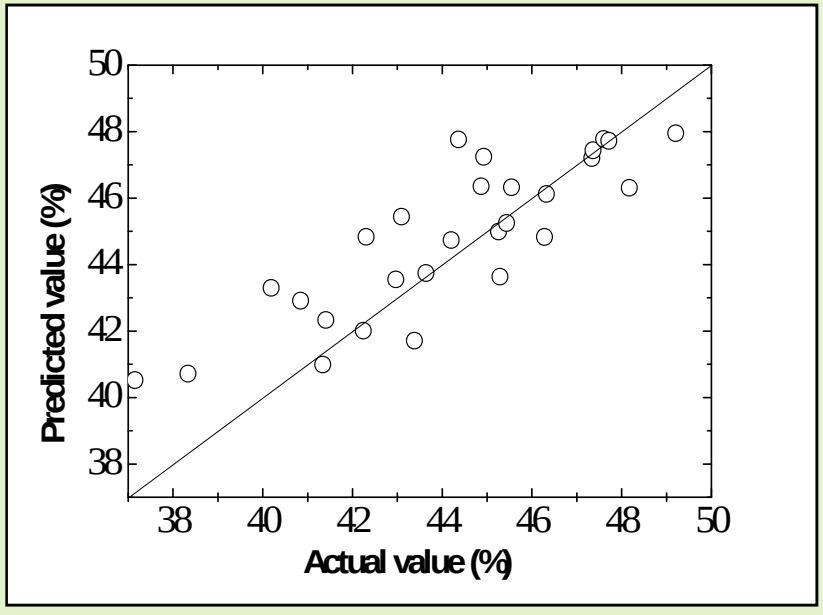
External prediction

Ratios of actual to NIR-predicted values



Free fatty acid ratio : R = 0.982

RPD = 5.158



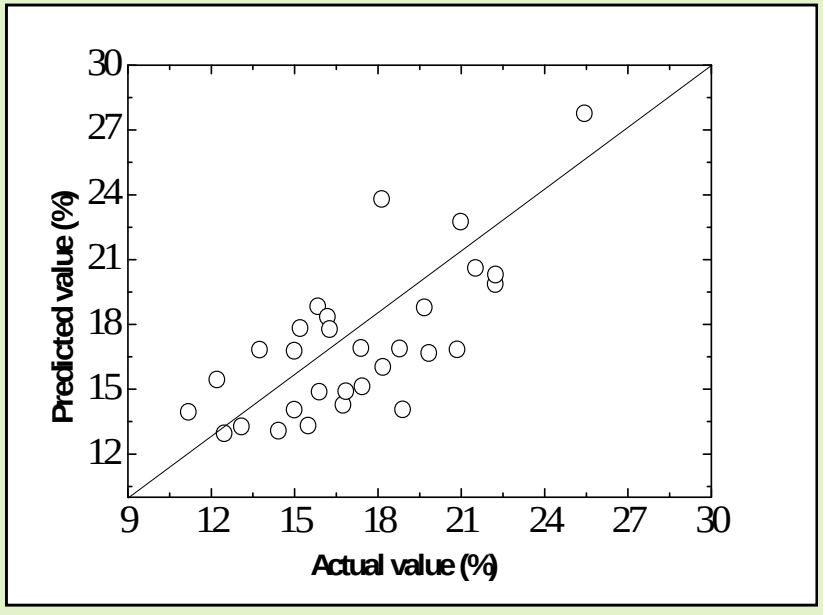
Oleic acid ratio : R = 0.857

RPD = 1.883

Results and Discussion

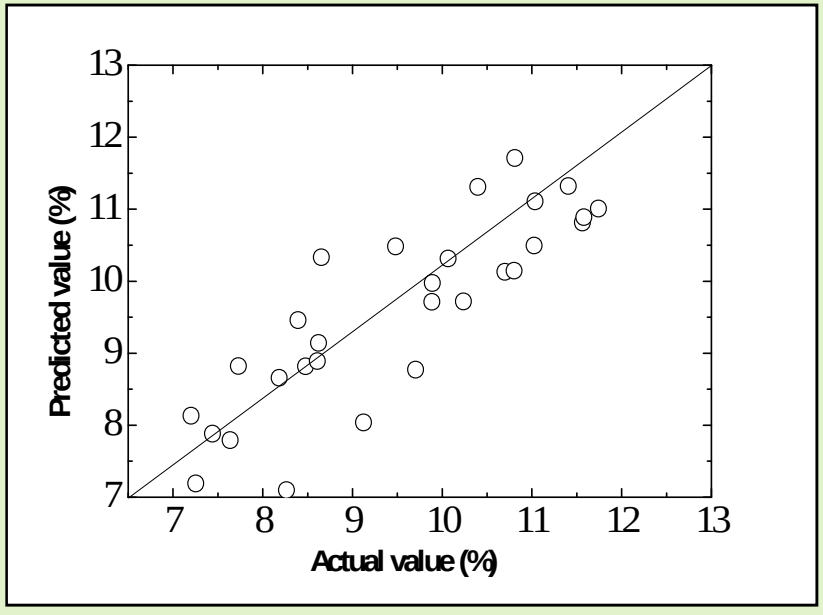
External prediction

Ratios of actual to NIR-predicted values



Linoleic acid ratio : R = 0.832

RPD = 1.791



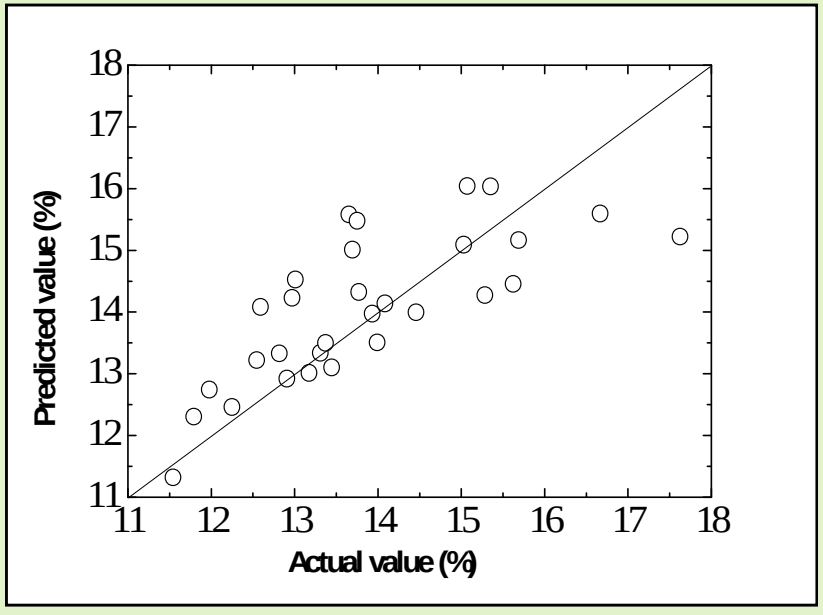
Behenic acid ratio : R = 0.839

RPD = 1.664

Results and Discussion

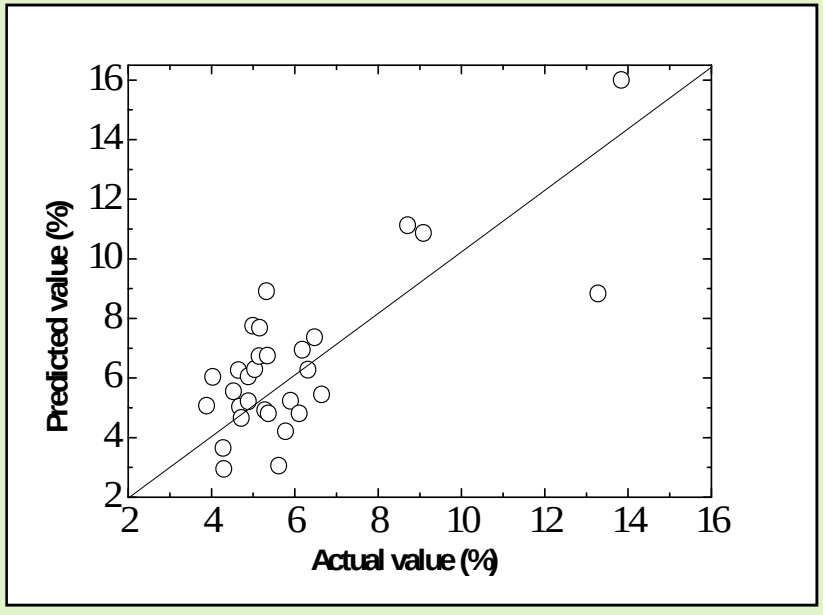
External prediction

Ratios of actual to NIR-predicted values



Palmitic acid ratio : R = 0.784

RPD = 1.480



Stearic acid ratio : R = 0.699

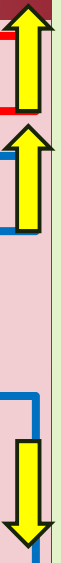
RPD = 1.251

Results and Discussion

External prediction

Summary pretreatment techniques in **"external prediction"**

Contents	Pretreatment	F	Prediction		
			SEP	SD	RPD_p
FFA	SM + 1De	4	0.830	4.281	5.158
C18:1	SM	12	2.007	3.780	1.883
C18:2	NM + 1De	12	2.236	4.004	1.791
C16:0	NM + 1De	10	0.899	1.331	1.480
C18:0	NM + 1De	13	0.927	1.160	1.251
C22:0	SM + 1De	12	1.073	1.785	1.664



good

poor

Results and Discussion

External prediction

The broad ranges of these compositions were the reasons that made all of these models had accurate prediction. Since palmitic acid and stearic acid had narrow range, so the prediction models were less accurate.

The less accuracy of palmitic acid and stearic acid could be sufficiently used by increasing the number and broader range of reference samples.

Conclusion

The results strikingly indicate that the reflectance NIRS has ability for estimating the fatty acid compositions and the free fatty acid content (FFA) in Pongamia Pinnata seed oil.

NIRS technique is a simple, non-destructive and rapid technique that can use for replace the conventional method.

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Thank you for your attention

Example for the calibration equation

$$\%\ \text{Arabinose} = b_0 + \sum_{i=1100}^{2500} b_i x_i$$

b_0 is y-intercept of regression model
b is the regression coefficient at wavelength i
x_i is absorbance
i is the wavelength

Materials and Methods

Spectral pre-treatment and calibration

Chemometrics

The chemical discipline that uses mathematical and statistical methods,

(a) to design or select optimal measurement procedures and experiments

(b) to provide maximum chemical information by analyzing chemical data.

Introduction

Near Infrared Spectroscopy (NIR) (Example)

Wavelength (nm)	Type of spectrum	Molecules
910	3rd Overtone of C-H	Protein
928	3rd Overtone of C-H	Oil
1450	1st Overtone of O-H	Water, starch
1685	1st Overtone of C-H	Aromatic
2050	Combination of N-H	Protein
2336	Combination of C-H	Cellulose