# Method development for expanding the application of Fourier-Transform Near-Infrared Spectroscopy (FT-NIR) in food samples

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# INTRODUCTION

Examination of the nutritional parameters of food products is a basic requirement of the production and distribution wheather we speak about qualitative or quantitative determination. The products and the manufacturers will have to meet the strict regulations and high standards of quality from the sides of authorities and consumer demands as well. Determination of physical and chemical parameters can be performed in classical methods in a laboratory and are fixed in the Codex Alimentarius Hungaricus.
These methods usually have high labour, time and cost demand, besides the chemicals used for the determination represent considerable environmental impact.
To reduce the strain, a decade ago so-called green chemical processes have been appeared as a new direction in the food analytics. The aim of these methods is to replace the classical meauserments or to offer an alternative. The above mentioned group includes the near infrared technology (NIR), which spreads as a non-destructive method more widely. The fields of application increase, in terms of components and samples, as the cost of a measurement is much less compared to conventional laboratory determinations.
The immediate response is important – if not the most important – benefit of the technology which is the most important requirement in the production plants and production lines. The simple design of the instruments allows at-line process control.
Due to the results of technical developments, handheld devices are now available in the market, which are ready to perform the analysis on live animals, this is called ’on-farm’ technique.
The rapid routine measurements help the manufacturers give proper information to the consumers, thereby the aim of the thesis was to develop calibration functions using near infrared spectroscopy, which may provide the basis of the determination of nutritional parameters in different food products.

# AIMS

The following goals had been defined before any analytical measurements were carried out. Determination of macrocomponents of food ingredients and products by using reference methods and thereafter working out estimating functions by the application of FT-NIR method (both cross and test validations used) in the forthcoming food matrices and target components:

- Bakery products: determination of protein-, fat- and carbohydrate contents
- Pastry products: determination of fat- and egg contents
- Cheeses: determination of protein- and fat contents
- *Brassica oleracea* samples: determination of protein- and fat contents
- *Fabaceae* samples: determination of protein- and fat contetnts
- *Fabaceae* samples: determination of gross energy content and population splitting to calibration and validation samples by WinISI and OPUS 6.5 softwares.

# MATERIALS AND METHODS

Bakery products, dry pasta, cheese, *Brassicaceae* samples and *Fabaceae* samples were used in this study.

For sample preparation a freeze dryer and a lab oven were used. Grinding was also used as further sample preparation to reach equal particle size. Sieving of the grinded samples was used to ensure the optimal homogenity.

For the protein content determination Dumas and Kjeldahl methods were applied. For the fat content determination in case of bakery products, dry pastas, *Brassicaceae* and *Fabaceae* samples the Hungarian Standard 20501-1:2007 method was applied, while the fat content of cheeses was determined according to the Hungarian Standard 2714/1-1989 and the Hungarian version of the EN ISO 1735:2004, the Hungarian Standard EN ISO 1735. The Hungarian standard 20500/4-87 provides two methods for the determination of egg content in pastry products. The decisive method which is based on the sterine content of the samples (both cholesterol and fitosterol) and the fast method which is based on the determination of the fat content of the samples. The basic of the decisive method is the Liebermann-Burchard reaction which resulting in bluish-greenish colour in the solution. The colour intensitiy is proportional to the sterine content of the sample.

This combined method has however several drawbacks; the extracted sterine content alters by time and thereby the extract processing is time-limited. The extraction efficiency of the standard fat content determination is rather doubtful. To achieve a better efficiency, I followed the process defined in the Hungarian Standard 20501-1:2007 standard for the fat content extraction in bakery products.

It should be emphasized that the calculation defined in the above standard method disregards the fat content of the flour used for pastry production which according to our research should be neglected. Therefore the developed equation presented in the Results section of the current thesis includes the flour fat content as well. This calculation gives the basis of the NIRS calibrations meant for egg-content in pastry products.

The gross energy in *Fabaceae* samples was determined with an IKA Werke Basic C2000 adiabatic bomb calorimeter (IKA Werka GmbH, Staufen, Germany). The powder

samples were pressed to produce pastilles. The determination took 20 minutes per sample. The gross energy content expressed in kcal/g.

The spectra were recorded with a BRUKER MPA™ FT-NIR spectrometer (Bruker Optik GmbH Ettlingen, Germany), the spectral range was set to 800-2500 nm (12500 – 4000 $cm^{-1}$). The reflection measurements were done using the sample wheel sample holder of the instrument but during the measurement of the legumes a rotation petri dish was used. The final spectra was resulted by an average of 32 subsequent scans (default setting in the software). The spectral resolution was 8 $cm^{-1}$. The background was recorded automatically with a gold coated integrating sphere. A lead-sulphite (PbS) detector was applied.

The results were evaluated with the OPUS 6.5 (Bruker Optik GmbH Ettlingen, Germany) and the WinISI II. (InfraSoft International, Port Matilda, PA, USA) instrument specific softwares.

During the calibration the following spectral pre-treatment were used: first derivative, second derivative, vector normalization (SNV), multiplicative scatter correction (MSC).

Principal component analysis was applied to detect the spectral outliers in case of the *Fabaceae* samples. Since the spectra were recorded with a Bruker MPA instrument, therefore the spectra need to be converted to appropriate format for the WinISI II. software.

Each calibration was done by OPUS 6.5 software Quant2 module by PLS regression.

# RESULTS

The protein content of the bakery products determined by the reference method was 8,2-16,2 m/m%. The calibration which had the root mean square error of cross-validation (RMSECV) was 0,25 m/m based on 178 samples. The fat content was determined from 64 samples and the range of the reference method was 1,2-31,1 m/m%. The estimation function had a RMSECV of 0,71 m/m%. During the determination of carbohydrate content one sample with higher carbohydrate content was identified. That sample was labelled as a chemical outlier. The range of the reference method was 0,9-11,5 m/m%. The calibration had a RMSECV value of 0,54 m/m%.

The fat content determination in pastry products was performed with 90 samples and the range of the reference measurement was 0,5-3,9 m/m%. The calibration had a RMSECV value of 0,15 m/m%.

For the determination of egg content in pastry products an indirect method was developed which was based on a calculated fat content of the different components in the pastry product. The following equation was created to calculate the egg content of pastry products:

$$m_1 * c_1 = x * m_2 * c_2 + (1000 - x * m_2) * d_3 * c_3,$$

| Abbreviation | Meaning | Measured or calculated value |
|---|---|---|
| $m_1$ | Dry weight content of the sample expressed in g | Measured |
| $m_2$ | Dry weight content of an average egg (g) | 12,77 g (calculated) |
| $c_1$ | Fat content of the sample expressed in m/m% | Measured |
| $c_2$ | Fat content of an average lyophilized egg (m/m%) | 38,2 m/m% (measured) |
| $c_3$ | Fat content of a pastry made without egg; normal, durum (m/m%) | 0,6 , 0,8 m/m% (measured) |
| $d_3$ | Dry weight content of a pastry made without egg | Measured |
| x | Egg content (piece) | Calculated |

The reference data was calculated with the above mentioned equation. The calibration based on 130 samples and the RMSECV value of the method was 0,5 piece egg. It can also be concluded that the method developed is not applicable to the analysis of pastry samples made without egg, that of low egg content (n<2 egg) or in case of adulteration.

For the determination of protein content of semi-hard and hard cheeses the FT-NIR method was applied and the average result based on 87 samples. The range of the reference method was 24,8-65,7 m/m%. The calibration had a RMSECV value of 1,17 m/m%. The range of the reference method was 19,1-55,6 m/m% in case of lipid content determination and the calibration had a RMSECV value of 0,50 m/m%.

For the determination of protein- and lipid content of *Brassicaceae* samples 63 samples were used. The ranges of the reference methods were 9,7-33,8 m/m% and 0,7-5,9 m/m% for the protein and the lipid content, respectively. The methods had a RMSECV values of 1,47 m/m% for protein and 0,5 m/m% for lipid. Nevertheless the statistical parameters of the methods are satisfactory, more samples need to be added to the calibrations.

During the analysis of the 120 *Fabaceae* samples PCA was applied. The analysis resulted that the granulated soybean samples must be rejected from the population. The ranges of the reference methods were 19,5-52,6 m/m% protein and lipid content respectively. The calibration had a RMSECV value of 1,44 m/m%. It can also be concluded that the calibration developed with 117 samples is not applicable to quantitative analyisis of the lipid content.

The method for the determination of gross energy content in *Fabaceae* samples is based on 80 samples. The range of the reference method was 4,15-5,42 kcal/g. The population splitted into calibration and validation sets using only spectral information. The calibration sets were defined with two independent softwares.

# NEW SCIENTIFIC RESULTS

1. The method of quantitative determination of macrocomponents in bakery products has been developed using Fourier-Transform Near-Infrared Spectroscopy (FT-NIR) for the first time. As a result, a robust estimating function has been worked out which is Hungarian Standard EN ISO/IEC 17025:2005 compatible and can easily be employed in accredited laboratories for the analysis of protein, fat and sugar content in various food matrices.

2. A new analytical method has been established for the determination of egg content in pastry samples by FT-NIR technique, which allows a simplier, a more robust and precise estimation than the current Hungarian Standard 20500-4:1987.

3. An FT-NIR method has been newly developed for the analysis of macrocomponents in vegetables. A robust estimating function has been established for the determination of protein and fat content in *Brassica oleracea* (*Brassicaceae* family) and also for the protein analysis of vegetables belonging to *Fabaceae* family.

4. Full protein content analysis has been developed for *Fabaceae* samples by FT-NIR technique for the first time. Comparing the FT-NIR method to the classical fat-, protein-, and carbohydrate content determinations, the FT-NIR method is faster, simplier, more economical and gives less load to the environment as well.

5. The joint use of OPUS 6.5 (Fourier-Transform Bruker equipment) and WinISI II (dispers FOSS NIR Systems) softwares tested for the first time together gives the possibility to separate calibrating and validating sample groups based on their spectral information.

# PUBLICATIONS

**Papers with impact factor:**

- **Tamas Szigedi**, Mihaly Dernovics, Marietta Fodor
  *Determination of protein, lipid and sugar contents in bakery products by using Fourier-transform near infrared spectroscopy* Acta Alimentaria 2011, 40 (Suppl.), 222-229.
  **$IF_{2011}$: 0,444**

- Marietta Fodor, Agnes Woller, Sandor Turza, **Tamas Szigedi**
  *Development of a rapid, non-destructive method for egg content determination in dry pasta using FT-NIR technique* Journal of Food Engineering 2011, 2, 195-199.
  **$IF_{2011}$: 2,414**

- **Tamas Szigedi**, Jozsef Lenart, Mihaly Dernovics, Sandor Turza, Marietta Fodor
  *Protein content determination in Brassica oleracea species using FT-NIR technique and PLS regression* International Journal of Food Science and Technology 2012, 47, 436-440.
  **$IF_{2011}$: 1,259**

- Jozsef Lenart, **Tamas Szigedi**, Mihaly Dernovics, Marietta Fodor
  *Determination of fat and protein contents in cheeses by FT-NIR spectroscopy* Acta Alimentaria 2012, 41, 351–362.
  **$IF_{2012}$: 0,475**

- **Tamas Szigedi**, Marietta Fodor, Dolores Perez-Marin, Ana Garrido-Varo
  *Fourier-transform near infrared spectroscopy to predict the gross energy content of food grade legumes* Food Analytical Methods 2013, 6, 1205-1211.
  **$IF_{2012}$: 1,969**

Poster or oral presentations in international and Hungarian conferences

- **Szigedi Tamas**: Method development for the determination of fat and egg content in pastry products by NIR technique.
  (XXIX. OTDK Conference, Food Science section, 2009. april 6-8., Gödöllő. Abstract, page 152. ISBN: 978-963-269-095-7)

- **Szigedi Tamas**, Dr. Fodor Marietta: Application of NIR technique for the determination of fat and egg content in pastry products.
  (Lippai János – Ormos Imre – Vas Károly Scientific Conference, 2009. october 29-30., Budapest)

- **Tamas Szigedi**, Mihaly Dernovics, Marietta Fodor: Method development for the determination of protein content in Brassica Oleracea samples using FT-NIR technique.
  7th Aegean Analytical Chemistry Days (AACD 2010), Lesvos, Greece,
  2010. september 28. - october 2.

- **Szigedi Tamas**, Dr. Turza Sándor, Dr. Dernovics Mihály, Dr. Fodor Marietta: Method development for the determination of protein content in Brassica oleracea species by FT-NIR technique.
  Hungalimentaria Conference, 2011. april 19-20., Budapest

- Fodor M., **Szigedi T**.: Application of FT-NIR technique in the field of quality assurance of food.
  Spectroscopic seminar, Budapest, 2011. september. 27-28.

- **T. Szigedi**, M. Dernovics, M. Fodor: Determination of egg content in dry pastas by FT-NIR technique
  'Pumpaya' Workshop, Kőszeg, 2011. május 6-7.

- **Szigedi Tamas**, Dr. Dernovics Mihaly, Dr. Fodor Marietta: Determination of gross energy content in Fabaceae samples using FT-NIR technique.
  TÁMOP closing conference, 2012. january 18-19., Budapest