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APPLICATION POSSIBILITIES OF NEAR INFRARED SPECTROSCOPY IN THE QUALIFICATION OF PORK, MEAT PRODUCTS AND LARD

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1. Background of research, objectives

Quick and effective evaluation of quality parameters of the different agricultural goods, among them animal products, namely meat and meat products is one of the important issues nowadays. Knowing the characteristics of a product means economical advantage for all the members of the product path, either in production, processing or marketing, nevertheless this is the principle of the process-control systems, and is essential for the accurate information of the customers. However, conventional qualification methods are precise, one part of them is costly, time-consuming, and requires labour (*e.g.* instrument based analytical methods), other part of them includes considerable subjective faults (*e.g.* organoleptic investigations).

According to these facts, there is notable demand for quick analytical methods that can be used effectively to describe the quality during the different phases of the animal product processing. Near infrared (NIR) spectroscopic method, already used successfully in quick routine analyses of agriculture and food industry, can provide a solution for this challenge.

Contrary to conventional chemical analyses, this quick method does not require reagents or solvents, thus reducing the cost of analysis (material, working hours, labour). Since no harmful waste is produced, the level of environmental pollution is very low. The NIR method is applicable for both qualitative (*e.g.* classification by types, quality parameters) and quantitative aspects (*e.g.* prediction of chemical composition, physical parameters). During qualitative analyses the differences between several groups are described by the spectral database, while in quantitative analyses also reference data (*e.g.* results of wet chemistry) are required beside NIR spectra. After exploring the relation of the reference and spectral database by a calibration model, there is a possibility for the prediction of parameters of further, independent samples. Since this is a correlative technique, the accuracy of the model depends pronouncedly by the applied calibration method.

Near infrared spectroscopic investigations were launched at the Kaposvár University, Faculty of Animal Science in 2005, when a FOSS NIRSystems 6500 spectrometer was installed owing to the NKFP 4/024/2004 research fund. At this time, as fourth-year undergraduate student, I had the possibility to join the research group. After getting the basic methodology experience, I took part in NIR analyses on rabbit meat and fatty goose liver, as part of investigations focused on foodstuffs with high added value originated from environment friendly animal production that serves healthy nutrition. Researches demonstrated in this dissertation were mainly based on the processing of the meat of mangalica, an autochthonous traditional fat-type pig.

Based on the above mentioned research background, the following objectives were defined for my study:

- Investigation of the applicability of the laboratory NIR technique for discrimination of pork originated from modern meat-type pigs (landrace, large white, crossbred) fattened by intensive large-scale production or from extensively reared mangalica pigs.
- NIR based classification of meat mixtures of different pig genotypes, and accordingly, the description of the impact of the varying composition of sausage-pastes on the NIR spectra in regard to the identification of the product.
- Focusing on the wide range of development possibilities, methodology study for identification of different pig meats with the open source R Project software package.
- Compilation of pork database for the future incoming NIR based composition-predictions of the laboratory, then development of NIR calibration with PLS regression for the estimation of protein and fat content of pork and pig meat mixtures.
- NIR classification of different frying fats (sunflower and rapeseed oils, pig and goose fat) heated on different temperatures during different time intervals, furthermore, description of heat treatment induced quality alterations of these frying fats.

2. Materials and methods

2.1. Samples

2.1.1. Pork, meat products and fatback

Fresh carcass meats and processed meat products (mixture, sausage-paste) of different pig genotypes were analyzed. During the investigation of meat identification, NIR spectra of 91 meat samples were recorded (27 mangalica, 26 landrace, 27 large white and 11 landrace × large white crossbred). Meat-type pigs were slaughtered at the average weight of 104 kg, while the average slaughter-weight of mangalica pigs was 157 kg. Identically, 1 kg sample was taken from all left loins (*m. longissimus dorsi*) at the area of last thoracic vertebra after 24 hours of chilling. Connective tissue parts were removed, thus these samples contained only the intramuscular fat. During the quantitative measurements, further landrace loin samples were added to the above mentioned sample set. Ground meat mixtures were produced using thigh of mangalica and landrace pork, where the mixing ratio of mangalica was 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 85, 90 and 95%. With adding landrace chop-bacon to the ground meat mixtures, sausage-pastes were prepared with typically mangalica or landrace meats. During the investigation of bacons, two separated regions of mangalica fatbacks were involved.
In case of necessity, grinding of samples was done with Retsch Grindomix 200, homogenization was done with IKA A11, samples were freeze-dried with a Christ Alpha 1-4 freeze-dryer.

2.1.2. Lard and frying fats

Lard (pig fat) purchased at retailment was heated during the first investigation, on 160, 170, 175, 180, 185, 190, 200 and 230 °C for eight hours daily, during four days. Sampling was performed at 8, 16, 24 and 32 hours of heat treatment. Rapeseed oil (Vénusz), sunflower oil (Tesco) and melted goose fat (Merian) used for the second investigation were purchased at retailment, while mangalica lard was melted from fatback, at 80 °C in a tempered waterbath. Samples were heated for 36 hours, consecutively on 140, 150, 160, 165, 170, 175 and 180 °C. Sampling of the hot frying media was performed at 4, 8, 12, 16, 20, 24, 28, 32 and 36 hours of heat treatment.

Samples were stored under N_2 gas, on 4 °C. The level of heat treatment was expressed by the heat-sum value (heat-sum [°C×h] = temperature [°C] × duration of heating [h]).

2.2. Reference measurements

Fat content (ether extract) of meat samples was determined by the Soxhlet method (crude fat content, ISO R-1443:1973). Nitrogen content was measured with Kjel-Foss Fast Nitrogen Analyzer after hydrochloric acid digestion. Protein content was calculated with multiplication of the nitrogen content by 6.25 (crude protein content, AOAC, 2000). Dry matter based composition data [DM%] were used in NIR calibrations. During the fatty acid analyses of the fatback samples, fatty acid methyl esters were produced after Folch-extraction, and these were determined with gas-chromatography.

Acid value (AV) of frying fats was determined with basic titration, according to 2.201 IUPAC (1987). Results were given in [mg KOH/g fat] units. The peroxide value (PV) was measured with titration, according to 965.33 AOAC (1995) and was given in [meq O_2/kg fat] units. The *p*-anisidine value was measured according to the 2.504 standard photometric IUPAC (1987) method, and values were given in [meq/kg fat]. Total oxidation (TOTOX) value was calculated by these two data: TOTOX = 2PV + *p*AV. Carbonyl-number determination was performed applying hydroxylamine-HCl titration method (Bhalerao *et al.*, 1961), and values were given in [meq/kg fat] units. Total polar material (TPM; [%]) was measured with column-chromatograpy (2.507 IUPAC, 1987). Ratio of dimer polymer triglycerides (DPTG; [%]) was determined according to Peled *et al.* (1975).

2.3. NIRS methodology

NIR spectroscopic measurements were performed with a FOSS NIRSystems 6500 spectrometer equipped with a Sample Transport Module (FOSS NIRSystems, Silver Spring (Laurel), MD, USA). WinISI II v1.5 spectral analytical software (InfraSoft International LLC, Port Matilda (State College), PA, USA) was used to operate the spectrometer. Spectra were collected in the

region from 1100 nm to 2500 nm at 2 nm intervals, and were recorded in log(1/*R*) format. Small Ring Cup or Camlock Cell with aluminium-plated reflector back (0.1 mm layer thickness) was used for reflectance or transflectance measurements. Spectra acquisition was performed on room temperature.

Quantitative evaluation of spectra was done with WinISI software. Global method was used during modified partial least squares (PLS) regression (Sinneave *et al.*, 1994). For qualitative analyses, the principal component analysis (PCA) and PLS discriminant analysis of WinISI (Murray *et al.*, 2001), GPLS method developed with the gpls package of open source R Project (Ding and Gentleman, 2004), and polar qualification system (Kaffka and Gyarmati, 1998) of PQS32 software (MetriNIR Research, Development and Service Co., Budapest, Hungary) were used.

2.3.1. Qualitative analyses

The methodological study for the classification of pork was performed in cooperation with Dr. György Kövér (Kaposvár University, Faculty of Economy Science, Department of Mathematics and Physics). This study was based on the R Project, an open source modular software package. Gpls (Ding, B.), KernSmooth (Ripley, B.) locpol (Cabrera, J.L.O.) and pls (Wehrens, R. and Mevik, B.H.) packages were used during the identification of the different pig genotypes. Fresh and freeze-dried samples were involved, no spectra preprocessing was applied before data analyses. Verification of the method was done with cross-validation and independent validation, and the dataset was analyzed also with WinISI software, as for control.

During the investigation of pig meat mixtures and sausage-pastes, reflectance NIR spectra of the samples were recorded. Meat mixtures were freeze-dried, homogenized and scanned repeatedly in reflectance mode. PLS discriminant analysis of WinISI program was used for the identification of groups, using first derivative (gap: 4, smooth: 4) preprocessed spectra.

NIR spectra recorded in transflectance mode were used for the classification of heated lard. The identification of the heat-sum groups was carried out using first derivative spectra (gap: 4, smooth: 4) and PLS discriminant analysis of the WinISI program. Verification was done using cross-validation.

In order to classify the heated frying fats, NIR spectra recorded in transflection mode were analyzed in the NIR Laboratory of Budapest Corvinus University, Faculty of Food Science, Department of Refrigeration and Livestocks' Products Technology. Heat-sum classes were identified with PQS32 software using point-method of polar qualification system. First derivative spectra (gap: 7, smooth: 5) in 1100-2500 nm wavelength interval were applied. Sensitivity value was calculated for the evaluation of the system (sensitivity = absolute polar distance of groups / sum of the standard deviations of the groups).

2.3.2. Quantitative analyses

Calibration equations were developed by Global PLS regression of the WinISI software, based on the samples with known composition. The wavelength interval of 400-2500 nm of the second derivative spectra (gap: 8, smooth: 6) was used. For preprocessing, Multiplicative Scatter Correction (MSC) and Standard Normal Variance (SNV) were applied. During the prediction of the fatty acid profile of pig fatback, Global PLS calibration was developed in WinISI, with the same settings as described above.

Transflection mode was applied during the measurement of the spectra of the heated lard and frying fats. WinISI program, PLS regression, Global method was used for prediction of the quantitative parameters. 400-2500 nm interval and second derivative (gap: 8, smooth: 6) spectra preprocessed with MSC were utilized.

Full cross-validation was used for testing the equations obtained in quantitative analyses. R^2, *SEC*, R^2_{CV} and *SECV* values were given to characterize the calibrations.

3. Results

3.1. Investigation of pork and meat products

3.1.1. Qualitative studies

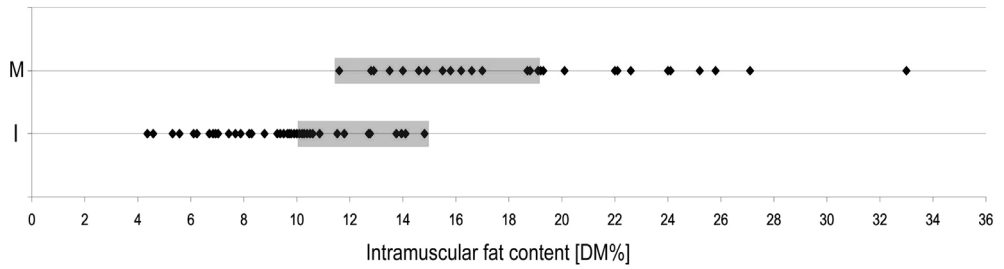
Classification model was developed in open source R Project for loin samples. Then, for testing our results, the spectral database of the meats was analyzed with WinISI, recommended software distributed by the manufacturer of the spectrometer. At last, the system was tested also with meat mixtures and sausage-pastes.

3.1.1.1. Classification of pig meat

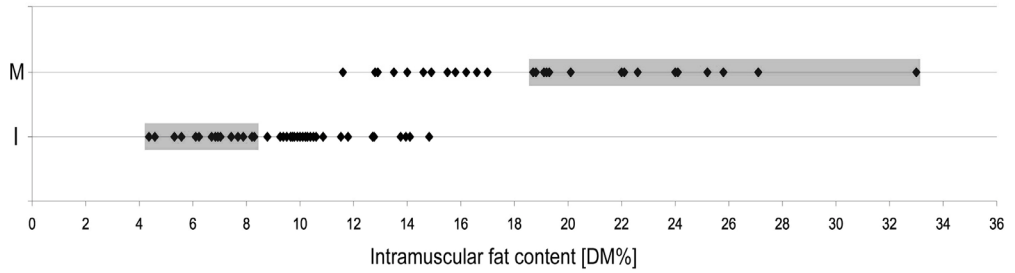
During our methodological study, we tried to solve a slightly simple challenge – *i.e.* classification of mangalica and meat-type pork. The samples of the three intensive meat-type pig breeds were merged and used as one single group, because of the identical chemical compositions. In the first trial, discrimination was run using all of the mangalica and intensive samples (n = 91). R Project based classification of the two groups was 100% effective during cross-validation, both when fresh or freeze-dried forms were applied. WinISI gave one fault for fresh samples, which means 99% success in cross-validation.

In order to explore and – if possible – ignore the clear effect of the considerable differences found in the chemical composition of the meats of the two groups, special sorting of samples with known laboratory data was applied during the classification. At first, 15 mangalica samples with the lowest intramuscular fat level and 15 most fattiest meat-type pork samples were involved into the calibration, and the remaining set was used for independent validation. Secondly, the fattiest 15 mangalica and 15 leanest meat-type pork samples were used for the calibration equation. This equation, generated on groups with very large differences in intramuscular fat content, was tested on the remaining 12 mangalica and 24 intensive pork samples having comparable fat content. The samples used for validation showed significant differences in fat content – according to the two genotype groups, however, due to the notable overlapping they were useful in testing the

accuracy of the discriminant equation generated on extremely different samples.



Trial setup by using overlapping groups for calibration (representing identical samples)
M: extensive mangalica; I: intensive meat-type
Box: samples used for calibration



Trial setup by using furthest groups for calibration (representing identical samples)
M: extensive mangalica; I: intensive meat-type
Box: samples used for calibration

A final discriminatory equation was generated using 20 randomly selected mangalica and 50 randomly selected intensive meat-type pork samples. The equation was tested with the remaining samples (7 mangalica, 14 meat-type). The results for these four trials are summarized in the table below. Since the results achieved with WinISI were practically the same, it can be declared, that the system classifies the samples not only by their intramuscular fat content, but the total multicomponent structure of meat is relevant and highly impacts the outcome.

Summarized results of R Project trials

Trial	Fresh samples				Freeze-dried samples			
	Cross-validation		Independent validation		Cross-validation		Independent validation	
	Nr. of factors	Hits	Nr. of factors	Hits	Nr. of factors	Hits	Nr. of factors	Hits
1	7	100%	-	-	5	100%	-	-
2	4	90%	4	97.2%	4	96.6%	4	94.4%
3	4	100%	4	91.7%	4	100%	4	94.4%
4	4	100%	5	90.5%	4	100%	5	95.2%

Trial 1: all samples involved for generating discriminator equation (*n*=27+64), no independent test
Trial 2: discriminator equation generated on overlapping groups (*n*=15+15), independent test with highly different groups (*n*=12+24)
Trial 3: discriminator equation generated on highly different groups (*n*=15+15), independent test with overlapping groups (*n*=12+24)
Trial 4: randomly selected samples used for discriminator equation (*n*=20+50), independent test with remaining samples (*n*=7+14)

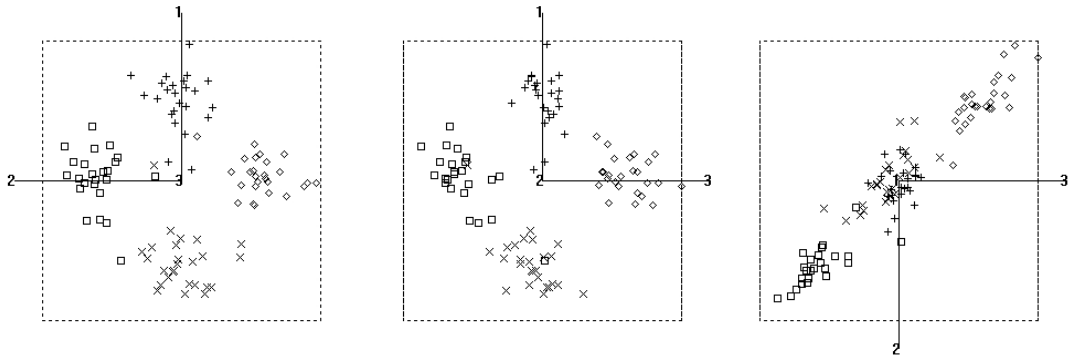
3.1.1.2. Classification of meat products

From the results obtained during classification of mangalica and landrace pig meat mixtures it can be seen, that NIR technique is applicable to detect even 5% changes in the mixing ratio of mangalica or meat-type pork.

Results for classification of different meat mixtures using different wavelength intervals of first derivative NIR spectra

Classification of groups containing different ratio of mangalica pork	400-2500 nm				1100-2500 nm			
	Fresh		Freeze-dried		Fresh		Freeze-dried	
	Nr. of factors	Hits %	Nr. of factors	Hits %	Nr. of factors	Hits %	Nr. of factors	Hits %
0-20% *vs.* 80-100%	3	100	1	100	4	100	1	100
0% *vs.* 10-100%	6	97.5	1	100	6	62.3	1	100
100% *vs.* 90-0%	5	87.5	2	100	3	75	2	100
0% *vs.* 5-100%	6	97.5	1	100	6	60	1	100
100% *vs.* 95-0%	6	74.8	5	100	3	67.3	5	100
100% *vs.* 95-70% *vs.* 60-40% *vs.* 30-5% *vs.* 0%	6	70.4	5	90.3	5	55.6	6	89
100-70% *vs.* 60-40% *vs.* 30-0%	6	94.7	5	95.2	6	93.6	4	90.3
100-80% *vs.* 60-40% *vs.* 20-0%	6	99.7	6	100	6	98	2	100

According to the results of further classification studies, the different mixtures can be discriminated easier using the total spectra, than using only separated wavelength regions characteristic for fat. Regarding the sausage-pastes, the ratio of successfully classified samples was 98% during the cross-validation, if the 400-1100 nm interval was applied.



Isolation of different sausage-pastes during PLS discriminant analysis fitted on 400-1100 nm wavelength interval
(□: mangalica delicate, ×: mangalica gyulai,
+: landrace delicate, ◊: landrace gyulai)

3.1.2. Quantitative studies

At first, calibration was developed using only loins of meat-type pigs, then the database was completed with loins of mangalica pigs. In the following trial, calibration was generated for the fat and protein content of mangalica and landrace thighs and the meat mixtures. Finally, the total sample set was merged – loins, thighs and mixtures – and common calibration was fitted on the NIR and laboratory data of the 181 samples.

In further trials we applied the software to search the characteristic wavelength regions that have considerable effects during the estimation of fat and protein content. It was unsurprising that regions of fat tended to be the most powerful at both constituents. According to this, calibrations were repeated using the narrow region of 1660-1760 nm. The results of this trial seemed to be comparable with that received when using the whole spectral region.

Results of NIR calibrations and cross-validations using different sample sets during estimation of fat and protein content (1100-2500 nm)

	Condition	Parameter	Factor	*SEC*	R^2	*SECV*	R^2_{CV}
Meat-type loin **(*n*=69)**	**Fresh**	**fat %**	3	0.83	0.917	0.98	0.883
		protein %	6	0.74	0.955	1.46	0.821
	Freeze-dried	**fat %**	5	0.52	0.968	0.67	0.947
		protein %	8	0.53	0.977	0.80	0.948
Meat-type + Mangalica loin **(*n*=96)**	**Fresh**	**fat %**	3	0.81	0.980	0.92	0.975
		protein %	4	0.95	0.973	1.29	0.951
	Freeze-dried	**fat %**	4	0.61	0.989	0.71	0.985
		protein %	9	0.50	0.992	0.76	0.983
Meat mixture thigh **(*n*=85)**	**Fresh**	**fat %**	6	0.80	0.994	1.26	0.986
		protein %	3	1.98	0.969	2.34	0.958
	Freeze-dried	**fat %**	7	0.35	0.999	0.48	0.998
		protein %	6	0.66	0.997	0.80	0.995
All samples miscellaneous **(*n*=181)**	**Fresh**	**fat %**	6	0.97	0.993	1.16	0.991
		protein %	5	1.90	0.972	2.12	0.965
	Freeze-dried	**fat %**	8	0.52	0.998	0.62	0.997
		protein %	11	0.65	0.997	0.86	0.994

SEC: standard error of calibration, R^2: coefficient of determination in calibration
SECV: standard error of cross-validation, R^2_{CV}: coefficient of determination in cross-validation

3.2. Investigation of fats

3.2.1. Heat treatment of lard

3.2.1.1. Qualitative analysis of heated lard

During our trial to classify the three heat-sum groups of the heated lard (below 2500 °C×h (*n*=8); between 2501 and 5000 °C×h (*n*=16); above 5501 °C×h (*n*=8)), the identification of the samples was less effective when the duration of heat treatment increased.

3.2.1.2. Quantitative analysis of heated lard

Intensive heat treatment caused marked changes in lard. Acid value (AV) increased in parallel with the length of heating, proportionally with the temperature. Contrary to AV, the peroxide value (PV) increased drastically only during the first eight hours of heating (approximately until 1300-1800 °C×h heat-sum value), then it started to decrease and stopped at the initial value. Carbonyl-number (CON) of lard practically remained at a

constant level within the first 16 hours (approximately until 3600 °C×h heat-sum value), then an expressed increase was detected.

Results of NIR calibrations and cross-validations using 800-2500 nm wavelength interval of second derivative spectra during estimation of heat-sum value and conventional quality parameters (*n*=32)

	Nr. of factors	*SEC*	R^2	*SECV*	R^2_{CV}
Heat-sum	2	653	0.87	807	0.81
AV	6	0.24	0.93	0.41	0.79
PV	1	22.5	0.48	24.1	0.43
TOTOX	1	51.9	0.26	57.1	0.13
CON	1	4.34	0.11	4.57	0.04

Regarding *p*-anisidine value (*p*AV), pronounced increase was observed during the experiment, when only treatments below 200 °C were taken into account (*i.e.* the temperature of practice). However, *p*AV decreased above 200 °C. Because of this considerable difference, separate calibration was developed for the group below 200 °C.

Results of NIR calibrations and cross-validations using different wavelength intervals of second derivative spectra during estimation of *p*-anisidine value (*n*=24)

*p*AV (< 200 °C)	Nr. of factors	*SEC*	R^2	*SECV*	R^2_{CV}
800-2500 nm	2	19.1	0.77	25.1	0.62
1100-2500 nm	4	13.7	0.88	24.3	0.65
1800-2500 nm	5	12.6	0.90	21.0	0.73
2000-2500 nm	5	11.8	0.91	19.4	0.77

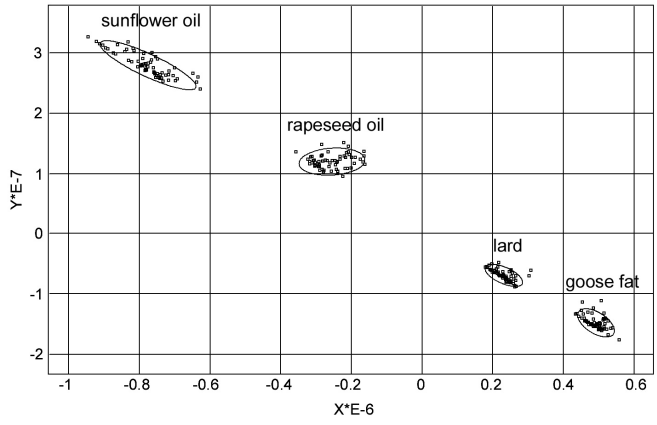
Consequently, TOTOX values, derived from PV and *p*AV having reverse shapings, were less informative in evaluation of the changes induced by heating.

3.2.2. Heat treatment of frying fats

3.2.2.1. Qualitative analysis of heated frying fats

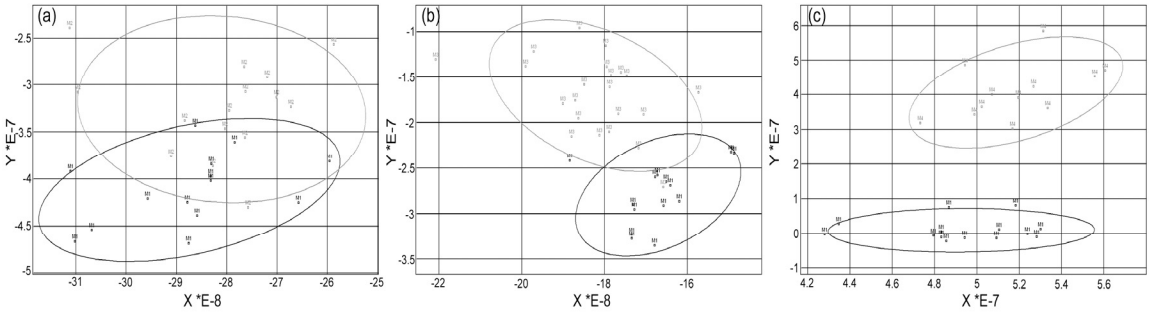
The first approach was aimed to identify the different heat-sum classes by PQS method, using the total sample set ((1) below 1500 °C×h (n = 56); (2) between 1501 and 3000 °C×h (n = 64); (3) between 3001 and 5000 °C×h (n = 76); (4) above 5001 °C×h (n = 56)). This approach had low success, showing that the type of fat or oil (in this setup these were subclasses) has a great impact and it can confuse the first step of identification. Secondly identification was run by the concept that fat type has more impact than the extent of heating. Thus, fat and oil type was taken as class, and heat-sum groups represented subclasses. Identification of primary classes (vegetable or animal origin) was the most successful in wavelength interval of 2400-2480 nm.

In the next step, the exact types of fats were determined within the primary classes. The best result for the discrimination between the two vegetable oils was found in the 2400-2460 nm interval, while the 1160-1200 nm range was the best to classify animal fats, with sensitivity values of 6.850 and 4.842, respectively. The optimum wavelength range obtained for animal fats (1160-1200 nm) was found to be a good screen for the type of the fats or oils in one step. Sensitivity values were always higher, when vegetable oils and animal fats were compared, showing the marked difference in the NIR spectra of the two primary groups (*i.e.* oils *vs.* fats).

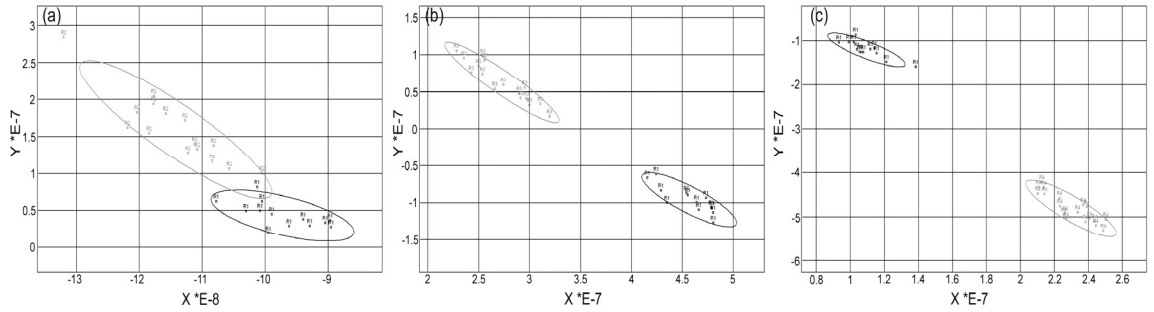


Location of the quality points of the investigated samples in the polar quality plane after a one-step classification for type (1160-1200 nm)

After fat and oil types (classes) were identified, repeated classifications and wavelength optimizations were run on heat-sum subclasses within all classes, respectively.



Location of the quality points of lard (pig fat) samples in the polar quality plane during classification for heat-sum groups
(a) 1 *vs.* 2: sensitivity = 0.962, wavelength interval = 1420-1492 nm
(b) 1 *vs.* 3: sensitivity = 1.437, wavelength interval = 2100-2124 nm
(c) 1 *vs.* 4: sensitivity = 3.218, wavelength interval = 2092-2204 nm



Location of the quality points of rapeseed oil samples in the polar quality plane during classification for heat-sum groups
(a) 1 *vs.* 2: sensitivity = 1.855, wavelength interval = 1424-1472 nm
(b) 1 *vs.* 3: sensitivity = 3.653, wavelength interval = 1916-1988 nm
(c) 1 *vs.* 4: sensitivity = 6.858, wavelength interval = 1128-1396 nm

The difference between the results of classification of oils or fats shows that quality deterioration of vegetable oils can be observed earlier than that of the animal fats. This might be due to the lower resistance of vegetable oils towards prolonged heating.

3.2.2.2. Quantitative analysis of heated frying fats

The table below summarizes results obtained for spectra based quantitative calibrations when 1100-2500 nm wavelength range was applied.

Results of NIR calibrations and cross-validations during classification for different parameters (1100-2500 nm)

	Fat type	*n*	*SEC*	R^2	*SECV*	R^2_{CV}	Factors
Heat-sum	**Total**	256	483	0.932	564	0.907	7
	Rapeseed	64	309	0.977	333	0.973	4
	Sunflower	64	297	0.974	371	0.960	2
	Pig	64	461	0.932	559	0.901	4
	Goose	64	376	0.953	533	0.907	5
AV	**Total**	256	0.17	0.936	0.20	0.918	8
	Rapeseed	64	0.13	0.968	0.18	0.936	3
	Sunflower	64	0.04	0.931	0.05	0.897	5
	Pig	64	0.12	0.273	0.13	0.215	1
	Goose	64	0.20	0.953	0.25	0.776	3
PV	**Total**	256	3.08	0.719	3.43	0.650	7
	Rapeseed	64	1.25	0.896	2.18	0.690	4
	Sunflower	64	1.74	0.949	2.10	0.927	8
	Pig	64	2.13	0.676	2.78	0.457	4
	Goose	64	3.75	0.547	4.86	0.251	4
CON	**Total**	256	56.6	0.279	59.2	0.209	6
	Rapeseed	64	45.9	0.786	81.8	0.334	3
	Sunflower	64	38.6	0.680	48.2	0.510	5
	Pig	64	13.6	0.070	15.5	0.015	1
	Goose	64	23.1	0.538	29.2	0.274	4
***p*AV**	**Total**	256	20.9	0.685	21.9	0.654	6
	Rapeseed	64	25.2	0.611	27.8	0.536	1
	Sunflower	64	28.0	0.068	28.7	0.042	2
	Pig	64	12.2	0.762	13.2	0.726	1
	Goose	64	685	0.931	9.54	0.869	5
TOTOX	**Total**	256	22.0	0.705	23.1	0.676	7
	Rapeseed	64	25.4	0.628	27.9	0.557	3
	Sunflower	64	24.4	0.445	30.9	0.128	2
	Pig	64	11.8	0.833	15.0	0.733	4
	Goose	64	9.68	0.911	15.7	0.771	6
TPM	**Total**	256	3.97	0.930	4.15	0.923	3
	Rapeseed	64	2.27	0.938	2.57	0.978	2
	Sunflower	64	3.91	0.920	4.02	0.917	1
	Pig	64	2.16	0.948	2.61	0.925	4
	Goose	64	5.31	0.752	5.45	0.744	1
DPTG	**Total**	256	2.62	0.293	2.35	0.263	3
	Rapeseed	64	2.19	0.047	2.24	0.022	1
	Sunflower	64	1.51	0.630	2.08	0.307	4
	Pig	64	2.66	0.046	2.78	0.220	1
	Goose	64	3.36	0.004	3.54	0.089	1

Calibrations for heat-sum value gave very good results. High correlations were described between the measured and predicted values of acid value (AV), both in calibration and cross-validation, except for pig fat, where results were unsatisfactory. Prediction of peroxide value (PV) was accurate when the whole dataset was involved into the calibration. Investigating the fat types separately, similarly good results were achieved for vegetable oils, but calibrations for animal fats were not acceptable. Carbonyl-number was also well predictable based on the spectral data in case of vegetable oils, but less accurate results were obtained for animal fats. In general, NIR based predictability of carbonyl-number seemed to be weak. Calibration for *p*-anisidine and TOTOX value was acceptable for practical use only in case of animal fats. Robust cross-validation results were described for total polar material (TPM), only goose fat was exceptional with lower scores.

The results for lard described above are contradictory to that of described also for lard in chapter 3.2.1.2. I must declare that the settings of these two investigations differed significantly, so they are not fully comparable, although both are focusing on lard. In my opinion, the differences of sample preparation and heat treatment could affect the conflict of results. Further studies are needed in order to achieve legitimate statements regarding calibrations for these parameters of lard.

3.2.3. Prediction of fatty acid profile of pig fatback

Fatty acid profile of mangalica fatback was predicted based on the 1100-2500 nm wavelength interval of NIR spectra. Satisfactory R^2 values were achieved in several cases for the fatty acids which were represented typically with high proportion within the fatback, but cross-validation tests provided weak results ($R^2_{CV} < 0.7$).

4. Conclusions, suggestions

Besides developing their NIR equipments, several hardware producers (FOSS, Bruker Optics, Büchi, Light Light Solutions, MetriNIR, Opotec, Perten, Unity) develop software for the processing of NIR spectra. There are also companies specialized for the thorough evaluation of the spectral data with their multifunctional chemometric program packages (*e.g.* CAMO Unscrambler). At the same time, there is a trend of researchers to focus on open source software, like R Project, that can be developed discretionarily.

Using R Project provides many advantages. The applied algorithm, the running settings, and the report forms of results can be adapted to the needs of the actual user. We want to utilize these advantages, which are most helpful in research applications, in order to process and evaluate the archived spectra of many types of meats of different lifestock (pig, cattle, broiler chicken, turkey, quail, pheasant, rabbit, diverse fish *etc.*), stored in the Product Qualification Laboratory of Kaposvár University.

According to the results of our study to discriminate the meats of the extensive mangalica or the intensive meat-type pigs, it can be stated that the system is classifying samples not only by fat content, but the total multi-component structure also has a great impact on results. However, further studies are required to identify the basic differences between meat from mangalica and other pigs. It can be reasonable to perform the classification trials with pretreatments, when the achieved results can be improved.

The comparison of the NIR spectra of the extensive autochthonous breed and intensive commercial breeds used in this investigation indicated some sharp differences in the fat regions (1210, 1720, 2304 and 2348 nm). These data are not presented because it is our conception that not only the total amount of fat but also the fat composition differed between the breeds of pigs we studied. An interesting point could be the investigation of the wavelength range of 500 and 577 nm, which is attributable to the main pigment in meat – myoglobin. Since mangalica pork has a darker meat compared to meats of commercial genotypes, this area could also be a basic factor in discrimination and needs to

be analyzed in the future. This possibility is also important, since the expanding market of mangalica products makes it necessary to use quick and accurate methods to identify meats and meat products.

There is a possibility to develop a quick scanning method for prediction of fat and protein content of meats and meat products. Thus, the time of the investigation (recording of the 100 nm range) reduces to some seconds, but the efficiency of prediction is comparable to that of reached with the total spectrum. However, it must be noted, that narrowing the range results in an increase of error level (*SEC* and *SECV*). One can decide by knowing the application field, if this increase of error is unessential and can be neglected in favour of time-saving or not.

Nowadays, the importance of foods prepared by deep fat frying is increasing. Modern culinary techniques use these vegetable oils – rarely animal fats – as frying medium, and at the same time these assist the formation of typical flavours of the product. However, it is well known, that organoleptic and nutritional quality of fats worsens during heating. Automatic wavelength optimization of the polar qualification system (PQS), used in our study for qualitative analysis of frying fats, provided the possibility to identify the fats or oils in a single step. This may help the identification of culinary fats by origin or types.

Since the conventional qualification of frying fats is time-consuming and needs solvents, we investigated the possibility to partly replace these methods with NIR technique. The increase of TPM value during heating – as the most important complex indicator of quality degradation of fats – was evaluated when the quicker deterioration of vegetable oils was demonstrated in contrast with animal fats. Because of the early increase of TPM level observed in vegetable oils, it is essential to develop such quick monitoring systems, which can be used in identification of unhealthy materials, overused substances.

Results achieved for animal fats regarding *p*-anisidine show the practical applicability of the method. The NIR based estimation of the level of this compound may be highly important for the practice; because *p*-anisidine is

toxic, a nontoxic quick assay or estimation may be valuable. At the same time, it must be taken into consideration, that NIR prediction is possible only if heating temperature is below 200 ºC.

It is recommended to use the transflector cuvettes in case of investigation of vegetable oils and animal fats on room temperature, where they are in liquid condition. After finishing this doctorate study, the equipment of the laboratory widened with a fiber optic testing probe. In our further studies we plan to compare this new technique with the former solution of cuvettes, based on simultaneous measurements with both tools.

It is needed to perform further studies to confirm the results achieved for the estimation of fatty acid profile of mangalica fatback, since the low cross-validation results ($R^2_{CV} < 0.7$) are not enough convincing. It is also possible that the investigation method applied for fatback samples is ineffective. The main reason of this might be the specular reflection of the fatback layers. Transmission measurement of slices with defined width might be reasonable because of the semi-transparent condition of fatback. Anyway, the applicability of NIR method is obvious by the presented results, but new measurements and data are needed to obtain better results and to come to more exact conclusions.

In general, the available mathematical-statistical methods and software backgrounds provide the possibility for achieving very precise and accurate calibrations. It is not rare to work out NIR based models that can predict the variance of the dependent variable very well, even almost totally. We must mention, however, that the obtained correlative results can never be taken with 100% reliability, thus, the method can never be certificatory and justifying. However, it is absolutely applicable for monitoring processes and for forming and promoting quick decisions. It can be applied successfully in fields, where a parameter must be checked, for which we have a NIR calibration with R^2 value over 0.9 and minimal error, but the laboratory reference measurement would need even more days. On the other hand, most customers are not interested about the exact composition of the purchased product but want to be sure that it fits to the group of desired goods with pre-

defined complex requirements. So there is an expressed need for better and better quick classifying systems, like NIR, in order to help monitoring-alert systems.

5. New scientific results

1) Classification of fresh and freeze-dried loin samples of extensively reared autochthonous mangalica and intensively fattened modern meat-type pig genotypes with PLS based discriminator equation resulted 100% success during the cross-validation, while the independent validation test gave 90.5% and 95.2% hit-rate for fresh and freeze-dried samples, respectively.

2) When discriminating meat mixtures containing extensive mangalica and intensive landrace meats, the detection of 10% mixing ratio of landrace meat was successful in 95% of cases, while 5% mixing ratio gave 92.2% hit-rate during the cross-validation, investigating first derivative spectra of freeze-dried samples.

3) Precise calibration with good cross-validation result ($R^2_{CV} > 0.96$) was obtained for fat and protein content of pork when 1100-2500 nm wavelength range was applied. Similarly precise results were achieved for the narrow interval of 1660-1760 nm ($R^2_{CV} > 0.95$), but the increase of standard error of cross-validation (*SECV*) indicates the lower level of accuracy.

4) I declared during PQS based qualitative analyses of frying fats, that the origin of fat or oil strongly affects the result of classification by heat-sum value. When applying the present trial setup, it is essential to identify the type of fats before identification of heat-sum classes. According to this, the best wavelength range for the separation of vegetable oils and animal fats was the 2400-2480 nm interval (sensitivity = 3.17, where sensitivity = absolute polar distance of groups / sum of the standard deviation of the groups). Within the vegetable oil or animal fat groups, the best classification for sunflower and rapeseed oils was found in the 2400-2460 nm range (sensitivity = 6.86), and 1160-1200 nm was the optimal interval for pig and goose fats (sensitivity = 4.84).

5) The wavelength interval of 1600-1200 nm is applicable for PQS based identification of heated rapeseed and sunflower oils, pig and goose fats, in a

single step, because sensitivity values higher than 4.61 were found in all cases of discrimination.

6) Classification of heat-sum classes with PQS provided acceptable results (sensitivity > 1) in case of rapeseed and sunflower oils, also from the early stage of heat treatment. This means that less than 1500 °C×h (approximately 9 hours of use on technological temperature) is enough to result in such changes of vegetable oils that can be detected with NIR based technique with high accuracy. Contrary to this, the early stage of heating (1500 °C×h) was detectable less successfully in case of pig and goose fats (sensitivity < 1).

7) Calibrations on heat-sum values provided good results during the quantitative analyses of heated frying fats, independently of the type of fat, and cross-validation results were high ($R^2_{CV} > 0.90$). In all cases except lard, high correlations were achieved between the measured and predicted data of acid value during the calibration ($R^2 > 0.93$), which was followed by a cross-validation with acceptable precision ($R^2_{CV} > 0.77$). Good results were achieved for the peroxide value of vegetable oils ($R^2 > 0.89$; $R^2_{CV} > 0.69$). Precision of calibration for *p*-anisidine value reached the level of applicability in case of pig and goose fat ($R^2_{CV} > 0.7$), while TOTOX value was acceptable only for goose fat ($R^2_{CV} = 0.77$). Precise, robust calibrations ($R^2 > 0.92$) and cross-validations ($R^2_{CV} > 0.91$) were achieved for the percentage of total polar materials of all types, except goose fat.

6. Scientific papers and presentations on the subject of the dissertation

6.1. Papers in foreign language

1. Bázár, Gy., Szabó, A., Romvári, R. (2010): NIR based quality control of frying fat samples by means of Polar Qualification System. Food Control, 21(7): 992-997. (IF: 2.463)

2. Szabó, A., Bázár, G., Locsmandi, L., Romvári, R. (2010): Quality alterations of four frying fats during long-term heating (conventional analysis and NIRS calibration). Journal of Food Quality, 33(1): 42-58. (IF: 0.600)

3. Bázár, Gy., Kövér, Gy., Locsmándi, L., Andrássy-Baka, G., Romvári, R. (2009): Identification of traditionally reared Mangalica pig's meat by near infrared spectroscopy using generalized partial least squares in open source R Project – a feasibility model study. Journal of Near Infrared Spectroscopy, 17(3): 119-125. (IF: 0.991)

4. Szabó, A., Bázár, Gy., Andrássy-Baka, G., Locsmándi, L., Romvári, R. (2009): A near infrared spectroscopic (NIR) approach to estimate quality alterations during prolonged heating of lard. Acta Alimentaria, 38(1): 97-106. (IF: 0.505)

6.2. Papers in Hungarian

1. Bázár, Gy., Romvári, R. (2009): Possibilities of near infrared (NIR) spectroscopy in livestock production (review) (in Hungarian). Állattenyésztés és Takarmányozás, 58(3): 265-280.

2. Kövér, Gy., Bázár, Gy. (2009): Graphical user interface for statistical evaluation of NIR spectra (in Hungarian). Acta Agraria Kaposváriensis, 12: 122-130.

3. Bázár, Gy., Kövér, Gy., Locsmándi, L., Romvári, R. (2008): Possibility for discrimination of Mangalica and intensive pork by means of near infrared spectra based discriminant analysis (in Hungarian). Animal welfare, etológia és tartástechnológia, 4: 730-737.

4. Kövér, Gy., Bázár, Gy. (2007): PLS (Partial Least Squares) regression and its application (in Hungarian). Acta Oeconomica Kaposváriensis, 1: 113-119.

6.3. Full proceedings of foreign conferences

1. Bázár, Gy., Kövér, Gy., Locsmándi, L., Szabó, A., Romvári, R. (2009): Detection of aliment adulteration by means of NIR spectroscopy – Feasible study based on open-source R Project. 14th International Conference on Near Infrared Spectroscopy, 7-16 November, Bangkok, Thailand, 527-531.

6.4. Posters published at foreign conferences

1. Bázár, G., Szabó, A., Romvári, R. (2008): NIR classification of frying fat samples by means of Polar Qualification System. 14th International Diffuse Refelctance Conference, 3-8 August, Chambersburg, PA, USA.

6.5. Full proceedings of Hungarian conferences

1. Bázár, Gy., Kövér, Gy., Romvári, R. (2009): Possibilities for near infrared spectroscopy based discrimination of pork originated from different pig genotypes (in Hungarian). 334. Tudományos Kollokvium, KÉKI, Budapest, március 6., Budapest, p. 6.

2. Szabó, A., Bázár, Gy., Romvári R. (2009): Conventional and NIR spectroscopic quality-description of heated frying fats (in Hungarian). 334. Tudományos Kollokvium, KÉKI, Budapest, március 6., Budapest, p. 5.

6.6. Presentations held at Hungarian conferences

1. Bázár, Gy., Kövér, Gy., Locsmándi, L., Szabó, A., Romvári, R. (2009): Detection of aliment adulteration by means of NIR spectroscopy (in Hungarian). MTA Kémiai Tudományok Osztálya Élelmiszeranalitika és -minőség Munkabizottság valamint a NIR Klub közös rendezvénye, Budapesti Corvinus Egyetem, november 3., Budapest.

2. Bázár, Gy., Szabó, A., Romvári, R. (2008): NIR spectra based classification of fraying fats by means of Polar Qualification System (in Hungarian). MTA Kémiai Tudományok Osztálya Élelmiszeranalitika és -minőség Munkabizottság valamint a NIR Klub közös rendezvénye, Budapesti Corvinus Egyetem, november 5., Budapest.

3. Kövér, Gy., Bázár, Gy. (2007): Possibilities of processing data derived from NIR spectrometer previos to PLS regression, by means of different statistical software packages (in Hungarian). VI. Alkalmazott Informatikai Konferencia, május 25., Kaposvár.