Thesis of PhD dissertation

NEAR-INFRARED SPECTROSCOPY FOR DETERMINATION OF RHEOLOGICAL PROPERTIES OF WHEAT SAMPLES

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1. Background and aims of the dissertation

Wheat qualification and determination of quality groups is a complex task. The farmers, producers and consumers have different demands regarding to the wheat quality when the use of wheat is for food, feed or energetic purpose. Therefore the quality could not be presented by one parameter, many parameters joined assessment should be carried out. The wheat quality determination is an assessment of physical, chemical (compositional) and technological properties. Besides analysis of microbiological status, possible contaminants complete the above mentioned measurements to give an overall picture about the items. Qualification is the procedure to consider the conformance according to the results. Besides basic parameters represented the purity, and hectolitre weight, moisture, protein and gluten content, Hagberg falling number presenting α-amylase activity, Zeleny sedimentation index, valorigraph water absorption, alveograph P/L deformity ratio and W deformation energy as rheological parameters determine the wheat quality and application. National and international methods and standards are available for determination of these parameters. The different countries have different criteria for these parameters (Győri and Győriné, 1998.)

The technological value of the wheat is determined mainly by the rheological characteristics. Generally, to measure rheological behaviour, a controlled, well-defined deformation or strain is applied to a dough over a given time and the resulting force response is measured. The general aims of rheological tests to obtain a quantitative description of the materials' mechanical properties, to obtain information related to the molecular structure and composition of the material, to characterise and simulate the material's performance during processing. The main techniques have traditionally been divided into descriptive empirical techniques and fundamental measurements. Descriptive empirical techniques are penetrometer, texturometer, consistometer, amylograph, farinograph, mixograph, extensigraph, alveograph. The main fundamental tests are flow viscometry, creep and stress relaxation (Dobraczyk and Morgenstern, 2003).

The valorigraph, instrument for dough analysis developed in Hungary according to the idea of Jenő Hankóczy, produced by Karl Brabender in 1927. The registered diagram (valorigram) represents the change of dough consistency through mixing. We can determine the water absorption and other parameters (dough development time, stability, softening) to determine the wheat baking quality.

The alveograph was produced by Marcel Chopin according to the idea of Jenő Hankóczy. During the alveograph test discs are formed from the wheat flour dough and these are stretched in two directions as a bubble. The procedure is followed by an alveogram and the following parameters could be read out: P (tenacity, mm), L (extensibility, mm), G (swelling index, ml), P/L ratio, W (deformation energy, 10^{-4} J).

Extensigprah is an uniaxial extension test. The test is suitable to determine the dough's resistance to extension and extensibility to give proper information about baking properties. The extensigraph parameters are: extensibility (mm), standard resistance to extension (the resistance at a constant extension of 5 cm) (BU), maximum resistance to extension (BU), and area under the curve (cm^2).

Determination of these characteristics needs high amount of samples; the analyses are time- and cost-consuming. In the last decades there is a higher demand for such technical methods, which are able to give the quality parameter with good accuracy in short time.

Near-infrared spectroscopy is rapid, non-invasive analytical tool, which is widely used in the different areas (e.g. agriculture, food and pharmaceutical industries). The near-infrared spectroscopy attends in agriculture by Karl Norris from the 1960's to predict the compositional data of crops. Nowadays we found solutions like analysis of crops on field at harvesting.

In near-infrared spectroscopy the interactions between the samples and the near infrared radiation are used: the rotation and deformation status of molecules are excited and the one part of the photons are absorbed, others transmitted and others have different ways.

The quantitative analysis needs calibration through this technique is indirect. The accuracy of the prediction is dependent from the accuracy of the reference analytical method, because the near infrared spectra evaluation is based on the results of a validated method.

The samples with many components have complex spectra, therefore it is possible that the local maximum of a group vibration of one component is at the same place than another and they are overlapping. Carry out proper analysis and evaluation needs statistical methods and chemometric softwares (Gergely, 2005).

The math treatments serve several purposes like removing noise, reduction of physical effects caused by different particle size distributions, enhancement of weak absorption bands, solution of the problem of overlapping peaks. Each of these transformations attempts to eliminate additive and multiplicative differences between spectra that are caused by variation in the partical size of particles rather than an actual variation in chemical composition. In model development multiples scatter correction, standard normal variate and derivatives. Principal component analysis or polar qualification system is used to screen the possible separation in the sample set.

The possible application of near-infrared spectroscopy in wheat quality determination is widely published in the literature. The wheat quality determination and their different aspects are in the focus of the studies: from the physical, chemical properties prediction, through technological properties prediction to possible contaminants detection. Calibration equations were developed to classify wheat based products and prediction their compositional data.

Many investigations are focused on the prediction of moisture and protein content by near-infrared spectroscopy (Williams and Sobering, 1993; Delwiche et al., 1998; Büchmann et al., 2001; Delwiche and Graybosch, 2003; Cozzolino et al., 2006; Başlar and Ertugay, 2011). These two parameters have important role at harvesting.

Other studies' aims is development of calibrations for baking properties prediction (Hrušková et al., 2001; Hrušková and Šmejda, 2003; Miralbes 2003 and 2004; Dowell et al., 2006; Jirsa et al., 2008; Mutlu et al., 2011; Arazuri et al., 2012). The developed models have different accuracy to predict farinograph, alveograph, extensigraph and mixograph parameters.

The following objectives of the work were drafted:

1. Investigation of rheological properties of samples from a small-parcel experiment and the possible effect of genotype and fertilization level on the NIR spectra and on the calibration models.
2. Investigation of rheological properties of samples from different growing area and the possible effect of growing area on the NIR spectra and on the calibration models.
3. Development of calibration equations by using different math treatments' combinations for prediction of valorigraph water absorption, alveograph W and

P/L value and extensigraph energy for extension (area under the curve; after 45, 90, 135 min) parameters.

2. Materials and methods

2.1. Samples

I could carry out the analyses on sample sets from different crop years. From 2008 I analysed samples from a small parcel experiment carried out in Látókép (experiment field of Centre of Agricultural Sciences, University of Debrecen), in which 18 wheat varieties were involved and applied 5 fertilization levels with control group to investigate the effect of genotype and fertilization. I analysed the following 16 varieties from the experiment: GK Öthalom, Lupus, Lupus*, Saturnus, Saturnus*, Sixtus*, Biotop, Biotop*, KG Széphalom, GK Kapos, GK Békés, GK Csillag, GK Petur, MV Suba, MV Verbunkos, MV Mazurka. The following fertilization levels were applied: control, 30 kg ha^{-1} nitrogen, 22.5 kg ha^{-1} P_2O_5 and 26.5 kg ha^{-1} K_2O and twofold, threefold, fourfold and fivefold of these amounts.

In my work I also analysed samples from 2009 and 2010 crop year, where the growing area was differ (independetly form the varieties). These samples were harvested in different regions of Hungary. In 2009 the areas were the followings: Gesztely, Látókép, Kisújszállás, Tápió, Tiszavasvári, Jánoshalma, Somogyszil, Csorvás, Körösszegapáti, Harta, Komádi, Dombóvár, Hajdúböszörmény, Pápa. In 2010 the areas were the followings: Harta, Somogyszil, Mezőkövesd, Jánoshalma, Nádudvar, Gesztely, Kapuvár, Tápió, Iregszemcse, Látókép, Csorvás.

2.2. Determination of wheat flours rheological parameters

The wheat samples were ground by LABOR MIM FQC 109 type laboratory mill (MSZ 6367/9:1989) with 250 μm sieve for valorigraph and exetensigraph tests. For alveograph test the wheat was ground by Chopin Laboratory Mill CD 1 with 160 μm sieve.

Determination of rheological properties was carried out by three tests. According to MSZ ISO 5530-3:1995 standard, valorigraph test by Valorigraf FQA 205 was carried out to investigate the water absorption of wheat-water dough.
The alveograph test was carried out according to AACC No. 54-30A method by Chopin alveograph. The measured parameters were the following: P value (tenacity) (mm), L value (mm), G value (swelling index) (ml), P/L value, W value (deformation energy) ($10^{-4}\cdot$ Joule).

Extenzograph test was also involved in the analysis of sample set from 2008. To determine the extensigraph properties of dough I used a Brabender extensigraph according to AACC No. 54-10 method. The studied extensigraph parameters were: extensibility (mm), standard resistance to extension (the resistance at a constant extension of 5 cm) (BU), maximum resistance to extension (BU), and area under the curve (cm^2). The extensigraph test was performed in three times: after a 45, 90, and 135 minute rest period.

2.3. Near-infrared measurements

FOSS Infratec 1241 Grain Analyzer was available for collecting spectra of wheat grains. The instrument works in transmission mode. It works in the 850-1048 nm region from wheat grains, with 2 nm pathlength, the number of subsamples were 2. The instrument was controlled by ISW v. 3.10 software and WH062008 model is available for prediction of some wheat quality parameter (moisture, protein, gluten content, Zeleny number, alveograph W).
For spectra evaluation I used WinISI II. v. 1.50 chemometric software.

2.4. Methods for evaluation of reference data and NIR spectra

2.4.1. Qualitative analysis of spectra – principal component analysis

In first stage of spectra evaluation I used PCA as a data reduction method by Win ISI II. software. I had different reasons for carry out PCA. PCA was performed to screen the possible effect of genotype and fertilization level in case of samples from the small parcel experiment, to screen the possible effect of the growing area and to screen the possible effect of crop year on spectra in a merged sample set. PCA was performed on raw and second derivative spectra.

2.4.2. Comparison of reference and NIR data

2.4.2.1. Math treatments

I chose SNV and SNV+D transformation and derivatives as math treatments. I used first and second derivatives, and in case of first derivative I applied two segment-gap set-ups (1-4-4-1 and 1-8-8-1). I worked with 2-2-2-1 set-up in case of second derivative. The raw and transformed spectra I was able to examine statistical parameters of calibration models of 12 combinations.

2.4.2.2. Modified partial least squares method (mPLS)

Development of calibration equations was carried out by mPLS regression method in WinISI II. software. I developed calibration models for prediction of water absorption by valorigraph, P/L and W by alveograph and where it was possible energy by extensigraph.

I evaluated the models according to its statistical properties. In one hand I looked at how many samples were used for development of the model compare to the original sample number and how many was taken out, which was noted in percent ratio. This ratio is not allowed to be higher than 10%. I gave the standard error of calibration (SEC) and the standard error of cross-validation (SECV). To characterise the accuracy of the model linear correlation coefficient (R^2) and RPD were given as well. R^2 shows how fit the comparison between the reference and NIR predicted data to a line with bias 0 and slope 1. RPD is a ratio of standard deviation of sample set to the SECV. The model has good accuracy when $0.7<R^2$ and $2.5<RPD$ (Nikolaï et al., 2007).

3. Results

3.1. Small-parcel experiment with different varieties in 2008

According to the results I can state, that the calibration models for prediction of rheological properties of samples from the small-parcel experiment have different accuracy. The PCA showed separate groups on (two varieties and samples of control group) the second derivative spectra, but does not show any in case of raw spectra. According to the PCA results samples belonging to one variety or fertilization level are close to each other, but there is no concrete separation.

To predict valorigraph water absorption I achieved a model with good accuracy after the PCA on raw spectra. Applying the spectra without any treatment and with SNV transformation I achieved models with R^2=0.87 and RPD=2.7. Mutlu et al. (2001) published models with the same accuracy.

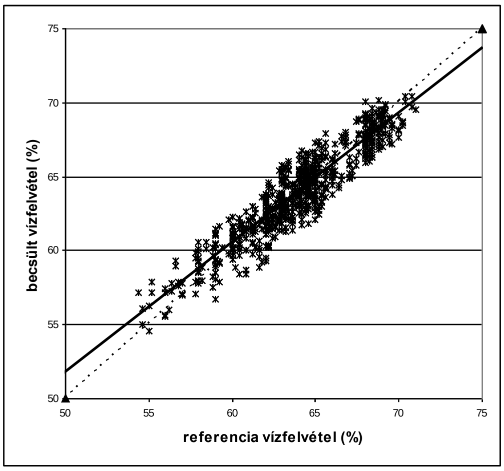
The removal of the two varieties according to the PCA on second derivative spectra has positive effect on the accuracy of the model predicting the alveograph P/L. To achieve R^2=0.80 I used spectra without any treatment and SNV+D transformation second derivative spectra, but the algorithm eliminated much samples (more than 10%). Publications show results with different accuracy, the most useful one is published by Arazuri et al. (2012) with R^2=0.86, RPD=5.9, but using second derivative spectra their model has lower R^2 and RPD (0.64 and 2.0, respectively) values. Dowell et al. (2006) had got model for prediction P/L with only informative prediction (R^2=0.47).

The removal of the two varieties according to the PCA on second derivative spectra has positive effect on the accuracy of the model predicting the alveograph W. Therefore the model using the spectra without any treatment and second derivative has 0.80 R^2 value, but because of the high SECV value, the RPD is lower and the accuracy of the prediction is not enough. Arazuri et al. (2012) achieved models with good accuracy (R^2=0.79, RPD=2.5) using second derivative spectra.

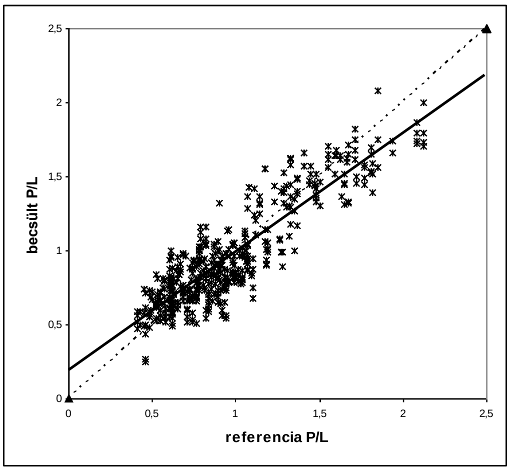
The models for prediction of extensigraph energy have different accuracy. The removal of the two varieties according to the PCA on second derivative spectra has positive effect only on the accuracy of the model predicting extensigraph energy E135, but it is still very low, the correlation is weak. Hruskova et al. (2001) made calibration models on two sample set and on merged sample set for predicting extensigraph parameters. They achieved same accuracy as me.

However, the removal of the two varieties according to the PCA on second derivative spectra has positive effect on the accuracy of some models, but I do not recommend excluding these varieties, because they are traditionally cultivated ones still now. The PCA showed some separation in case second derivative spectra, but neither the varieties nor the fertilization levels do not show direct, separate groups.

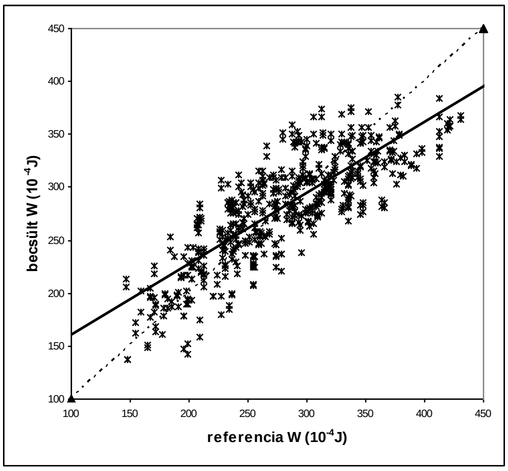
The noted calibration models are presented on Figure 1.



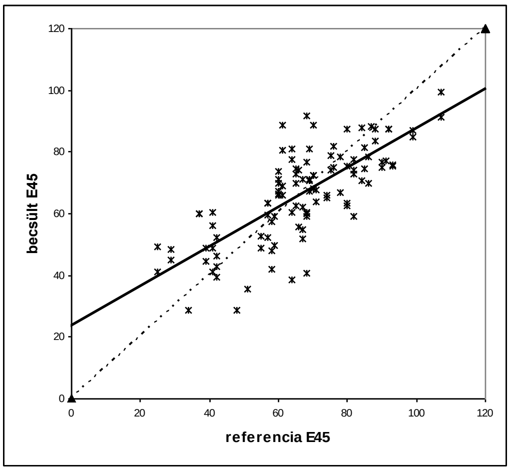
Original sample set, without treatment, raw spectra (0-0-1-1), R^2=0.87



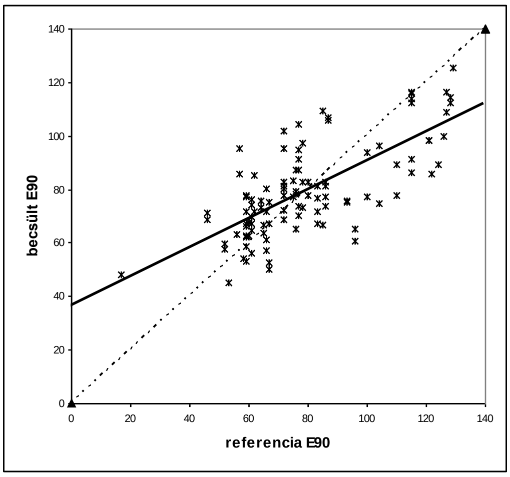
After PCA on second derivative spectra, exclusion of Lupus and GK Öthalom, without treatment, second derivative spectra (2-2-2-1), R^2=0.80



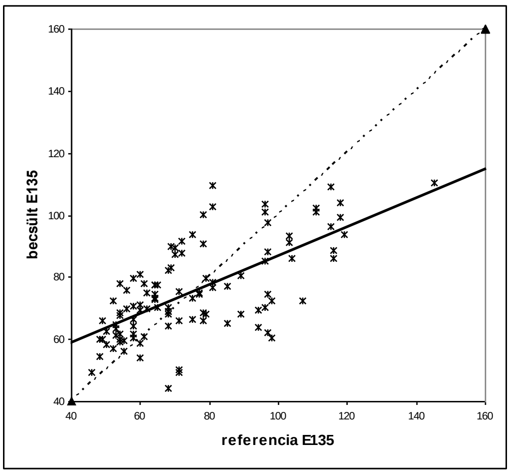
After PCA on second derivative spectra, exclusion of Lupus and GK Öthalom, without treatment, second derivative spectra (2-2-2-1), R^2=0.80



Original sample set, without treatment, first derivative spectra (1-8-8-1), R^2=0.67



Original sample set, without treatment, raw spectra (0-0-1-1), R^2=0.54



Original sample set, without treatment, first derivative spectra (1-4-4-1), R^2=0.48

Figure 1. Comparison between reference rheological values and NIR predicted values in case of small-parcel experiment in 2008.

3.2. Experiment with varieties on different growing areas in 2009

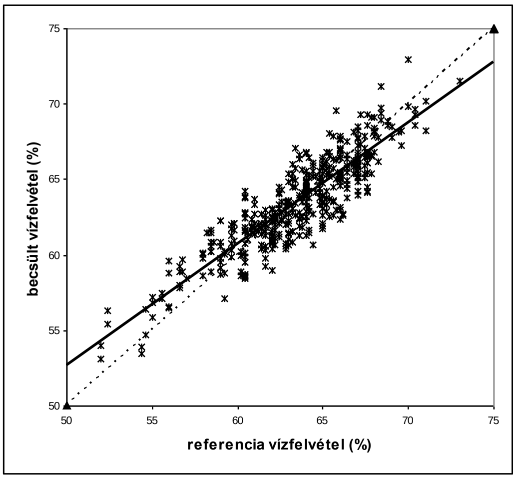
Calibration models were developed for prediction of valorigraph water absorption and alveograph P/L and W value on a sample set from 2009. The PCA showed separate groups in both cases (PCA on raw spectra and on second derivative spectra), thus I removed these samples to carry out again the model development. According to the results of PCA I can say that although there was growing area which samples formed separate groups, but the growing area does not show significant effect neither on raw spectra nor second derivative spectra.

The removal of samples according to PCA on raw spectra resulted more reliable models for prediction of valorigraph water absorption. I used spectra without any treatment and second derivative to achieve R^2=0.82 and RPD=2.2 which gives good accuracy for prediction of this parameter. Dowell et al. (2006) published result for this parameters with the same instrument and their model's R^2=0.65.

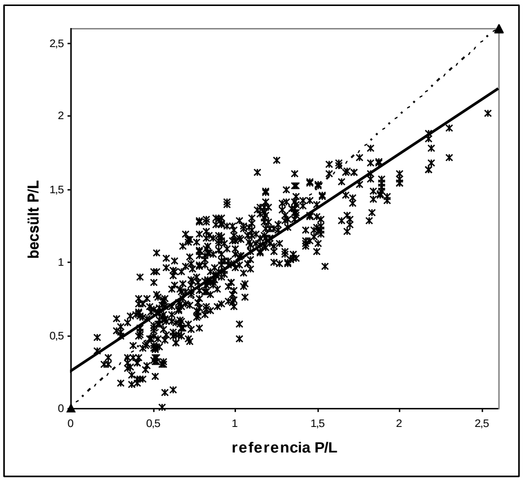
The removal of the samples according to the PCA has negative effect on the models' accuracy for prediction of alveograph P/L, thus the model development on spectra with SNV transformation and first derivative (1-4-4-1) gives R^2=0.75, but RPD=1.95 which means only informative prediction. The model's correlation coefficient is the same as for Arazuri et al. (2012), but the RPD is low.

The removal of samples according to PCA on raw spectra has positive effect on the accuracy of the models for prediction of alveograph W. The models using spectra without any treatment and SNV transformation have R^2=0.79 and RPD=2.18 and 2.15. The models' accuracy is moderate. Dowell et al. (2006) achieved R^2=0.69 with Savitzky-Golay first derivative spectra.

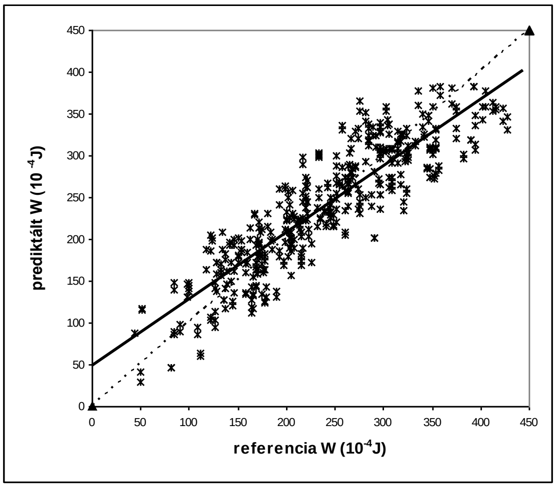
The noted calibration models are presented on Figure 2.



After PCA on raw spectra, exclusion of samples from Kisújszállás, without treatment, second derivative spectra (2-2-2-1), R^2=0.82



Original sample set, SNV, first derivative spectra (1-4-4-1), R^2=0.75



After PCA on raw spectra, exclusion of samples from Kisújszállás, without treatment, first derivative spectra (1-4-4-1), R^2=0.79

Figure 2. Comparison between reference rheological values and NIR predicted values in case of sample set in 2009

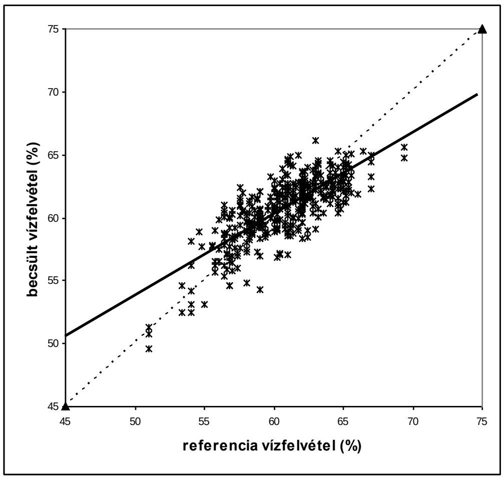
3.3. Experiment with varieties on different growing areas in 2010

Calibration models were developed for prediction of valorigraph water absorption and alveograph P/L and W value on a sample set from 2010. The PCA on second derivative spectra showed separate groups, thus I removed them from the sample set to carry out again the model development. According to the PCA the growing area does not have significant effect on the spectra; the samples belonging to the same growing area are not form separate groups.

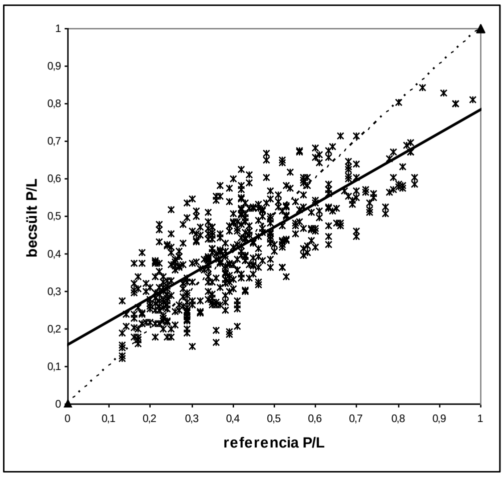
The removal of sample set according to PCA on second derivative spectra has positive effect on the models' accuracy for prediction of valorigraph water absorption. Using SNV transformed second derivative spectra I achieved model with higher R^2=0.66 and RPD=1.6 than for the original sample set, but the accuracy is low. The model'a accuracy is lower than in the literature (Mutlu et al., 2011).

For this sample set the removal of the samples according to PCA on second derivative spectra has no positive effect on the models' accuracy neither for prediction of alveograph P/L nor W, they are lower than earlier. Compare my results to the literature a model for prediction P/L has R^2=0.47 has lower accuracy, but my one is still not reliable for prediction this parameter.

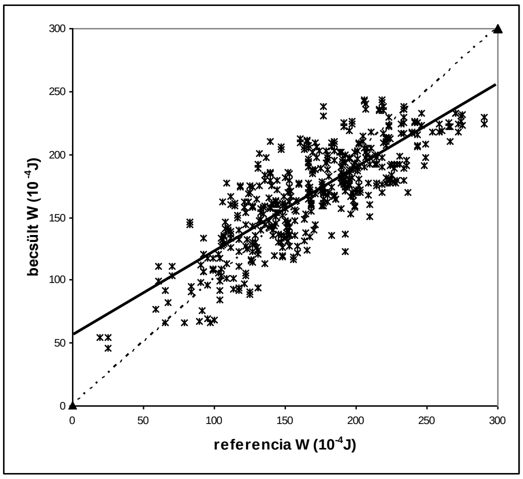
The noted calibration models are presented on Figure 3.



After PCA on second derivative spectra, exclusion of samples from Csorvás and one part of Látókép, SNV, second derivative spectra (2-2-2-1), R^2=0.66



Original sample set, without treatment, first derivative spectra (1-4-4-1), R^2=0.62



Original sample set, SNV, first derivative spectra (1-4-4-1), R^2=0,67

Figure 3. Comparison between reference rheological values and NIR predicted values in case of sample set in 2010

3.4. Experiment with varieties on different growing areas in 2009 and 2010

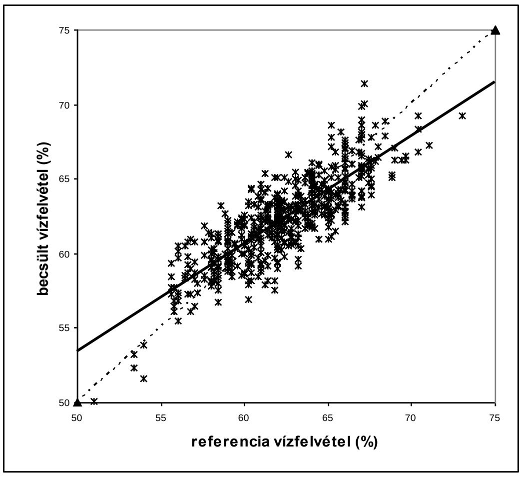
I developed calibration models for prediction of valorigraph water absorption, alveogrpah P/L and W of the merged sample set. PCA on second derivative spectra show separate groups (same as for samples in 2010), thus I removed them from the sample set and carry out again the model development.

I do not achieved models for prediction of water absorption with good accuracy. However the removal of samples according to PCA on second derivative spectra has positive effect on the R^2, but the model is still not reliable for prediction. Dowell et al. (2006) had got same results R^2=0.65, which one 0.66 for me.

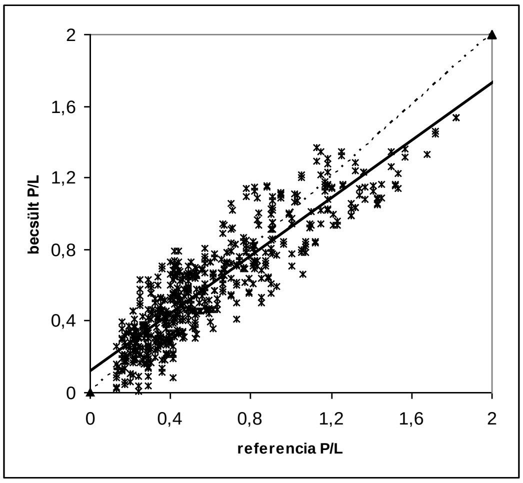
In case of alveograph P/L the regression eliminated more than 10% of the samples, but the R^2=0.81 and RPD=2,2 with using the original sample set, spectra without treatment and first derivative (1-4-4-1). The model's statistical parameters are better than written in the literature (Miralbes, 2004; Dowell et al., 2006).

In case of alveograph W the removal of samples according to PCA on second derivative spectra resulted models with R^2=0.76 and RPD=2,0 and 1,9 respectively for spectra without treatment and SNV transformed second derivative spectra. The models' statistical parameters are lower than for Arazuri et al. (2012), but better than Dowell et al. (2006) and Jirsa et al. (2008).

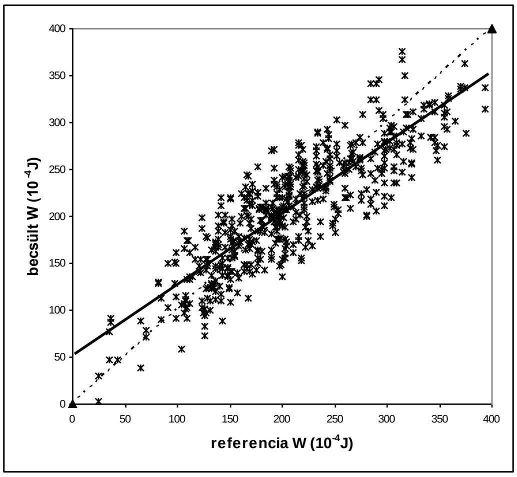
The noted calibration models are presented on Figure 4.



After PCA on second derivative spectra, exclusion of samples from Csorvás and one part of Látókép, SNV, second derivative spectra (2-2-2-1), R^2=0.73



Original sample set, without treatment, raw spectra (0-0-0-1), R^2=0.81



After PCA on second derivative spectra, exclusion of samples from Csorvás and one part of Látókép, without treatment, second derivative spectra (2-2-2-1), R^2=0.76

Figure 4. Comparison between reference rheological values and NIR predicted values in case of the merged sample set

4. New scientific results

1. I developed new method for prediction of valorigraph water absorption of wheat samples by near-infrared spectroscopy, which is able to determine this breadmeking quality parameter. Through the model development procedure I came to the conclusion that I have got models with good accuracy both using spectra without math treatment, spectra transformed by SNV, without derivation and SNV

transformed second derivative spectra, so the application of math treatments are not influenced the accuracy. However the removal of samples sets according to principal component analysis has higher influence on the accuracy.

2. I developed new method for prediction of alveograph P/L deformation ratio of wheat by near-infrared spectroscopy. The removal of samples set according to PCA has positive influence on models' statistical properties only in one case. I can state that models developed by raw spectra and second derivative spectra without math treatment gave the same accuracy than the models developed by SNV transformed first derivative spectra, thus the PCA has higher influence than math treatments. However the correlations are weak, only informative prediction could be carried out.

3. I developed new method for prediction of alveograph W (deformation energy) of wheat by near-infrared spectroscopy. The alveograph W could be predicted with coarse accuracy by this method. The removal of sample sets according to PCA has positive influence on the models accuracy. According to my results the most suitable model was developed by first (1-4-4-1) and second derivative spectra.

4. I developed new methods for prediction of extensigraph energy values (E45, E90, E135) of wheat by near-infrared spectroscopy. The models for prediction of energy values belonging to 45, 90, 135 min energy values has bad accuracy, no prediction could be carry out.

5. Scientific results in practice

In my work I developed calibration models for predicting wheat rheological parameters (water absorption, alveograph P/L and W, extensigraph energy values).

The model for prediction of water absorption gives the most reliable accuracy. The model could be applied in preliminary and on field wheat qualification, while the analysis does not need grinding, the water absorption determining baking quality could be predicted by whole grains.

The models for predicting alveograph P/L and W could be promising for agriculture, milling and baking industry. An approximate characteristic could be given about the processing behaviour and the utilization purpose.

The models for predicting extensigraph energy values (E45, E90, E135) have not enough accuracy to carry out any prediction.

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