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Research Article

# Determination of protein and gluten quality-related parameters of wheat flour using near-infrared reflectance spectroscopy (NIRS)

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**Abstract:** Wet chemistry analyses done for quality control purposes in wheat and flour processing are time-consuming. Near-infrared spectroscopy (NIRS) as an alternative technology to conventional methods allows us to obtain results in a few seconds. In this study, NIRS was used in the development of calibration models for protein, wet and dry gluten contents and Zeleny sedimentation of flours from the grinding of 120 varieties of bread wheat collected from different regions of Turkey. Therefore, spectra in the range of 1100 to 2500 nm of the flours were acquired by scanning with the NIRSystems 6500 monochromator. Multiple linear regression (MLR) and partial least squares (PLS) regression were applied to the spectral data in log 1/R, and first derivative and second derivative of log 1/R formats. Reasonable results were obtained for protein, wet and dry gluten contents, and Zeleny sedimentation with r = 0.985, 0.976, 0.953, and 0.924, respectively.

**Key words:** Gluten, NIRS, protein, Zeleny sedimentation

## Yakın Kızılötesi Spektroskopisi (NIRS) kullanarak buğday unlarının protein ve glutenle ilgili kalite parametrelerinin belirlenmesi

**Özet:** Buğday ve un işleme teknolojisinde kalite/kontrol için yapılan kimyasal analizler oldukça zaman alıcıdır. Klasik metotlara alternatif bir teknoloji olan yakın kızılötesi (NIR) spektroskopisi sonuçların birkaç saniyede elde edilmesini sağlar. Bu çalışmada, Türkiye'nin farklı bölgelerinden bir araya getirilen 120 çeşit buğday öğütüldü ve unlarda protein, yaş gluten, kuru gluten ve Zeleny sedimantasyon değerlerinin kalibrasyon modellerini geliştirmek için NIR spektroskopisi kullanıldı. Bunun için, monokromatör NIRSystem 6500 ile taranarak unların 1100 nm ile 2500 nm aralığındaki spektraları elde edildi. Unların log 1/R'deki spektral verisi ile log 1/R'nin 1. ve 2. türevlerine Çoklu Lineer Regresyon (MLR) ve Kısmi En az Kareler (PLS) uygulandı. Protein, yaş gluten, kuru gluten ve Zeleny sedimantasyon parametreleri için korelasyonları sırasıyla r = 0.985, 0.976, 0.953 ve 0.924 olan sonuçlar geliştirildi.

**Anahtar sözcükler:** Gluten, NIR, protein, Zeleny sedimantasyon

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## Introduction

The quality of wheat flours can be defined for several parameters including protein, moisture, gluten, sedimentation, enzyme activity, and rheological properties, none of which serves as adequate by itself. Bread making potential is derived largely from the quantity and quality of a flour's protein (Hruskova and Famera 2003). Although total protein content is the primary factor in characterizing wheat flour, it is not enough. Therefore, it is necessary to determine the gluten properties as well as protein quality. Moreover, wheat flour's strength is based on the gluten quality and content (Jirsa and Hruskova 2005).

Gluten, the protein component of flour which gives the dough elasticity and strength, can be defined as the rubbery mass that remains when wheat dough is washed to remove starch granules and water-soluble constituents. Gluten plays a key role in determining the unique baking quality of wheat by conferring water absorption capacity, cohesiveness, viscosity, and elasticity on dough (Wieser 2007). Generally, the higher a flour's protein content, the higher the gluten formation. During dough mixing, wheat flour is hydrated and the gluten proteins are transformed into a continuous cohesive viscoelastic gluten protein network.

Knowing the protein and gluten content is also not enough to fully characterize wheat flour. Therefore, it is useful to measure sedimentation value. The sedimentation value indicating correlation with gluten content, gluten quality, baking quality and loaf volume depends on the protein composition and is mostly correlated to the protein content (Hruskova and Famera 2003).

The baking industry requires flours to have defined quality characteristics including protein content, wet and dry gluten, and rheological properties. This supposes that the flour milling industry must complete many physical and chemical analyses, with consequent economic and time-related costs (Miralbes 2004). Therefore, NIR spectroscopy (NIRS), which is one of the various technologies for the evaluation of wheat flour quality, has become more popular and is attracting more attention from food researchers, recently (Ertugay et al. 2007). NIRS offers a number of important advantages over traditional chemical methods. A major advantage of this technique is that generally no sample preparation is necessary, and it can be carried out on the production line since the analysis is very simple and very fast. Furthermore, this technique allows various constituents to be determined simultaneously and preserves the sample after the measurement for further analysis. It has been widely used to predict the concentration of various constituents in food, wheat flour quality parameters including proteins (Norris 1978; Osborne et al. 1982; Delwiche 1995, 1998; Ertugay et al. 2007; Jirsa et al. 2008), and rheological characteristics (Williams et al. 1988; Hruskova et al. 2001; Hruskova and Famera 2003; Hruskova and Smejda 2003; Jirsa and Hruskova 2005).

Previous researchers (Pawlinsky and Williams 1998; Miralbes 2003, 2004; Ertugay et al. 2007; Jirsa et al. 2008) have shown NIRS to have potential for predicting wet and dry gluten contents and Zeleny sedimentation of wheat flours, although the applicability has mostly been inadequate for commercial use.

The purpose of the current study was to utilize the potential of NIRS for the determination of protein, wet and dry gluten contents and Zeleny sedimentation of wheat flour. It was also intended that quality analyses of bread wheat flours in Turkey could be determined by means of NIRS and a database could be created related to this technique to be used in local and international scientific research.

## Materials and methods

### Flour

One hundred twenty bread wheat samples from the 2007 crop year were obtained from different regions of Turkey (Sakarya, Eskişehir, Uşak, Afyon, Isparta, Konya, Mardin, Şanlıurfa, Siirt, Diyarbakır, and Erzurum). Their cultivars are from Pamukova-97, Tahirova-2000, Orhangazi, Sapanca, Kaynarca, Kate A-1, Momtchil, Nurkent, Pehlivan, Gönen-98, Basribey, and Adana-99, commonly grown in Turkey. Clarified wheat grains were conditioned to a moisture level between 14% and 15% depending on kernel hardness according to AACC method 26-10 (AACC 2000). Flour samples were obtained by milling wheat on a Buhler laboratory mill (model MLU-202) and were sieved with a 212 µm sieve. The samples were selected primarily based on their protein content (from 8.23% to 19.49%) and were expected to result in a wide range of bread quality parameters.

It is important to select samples with uniform distribution of the constituent or constituents to be determined because accuracy of prediction depends on the range of the flour quality parameters. The samples have a large constituent range and they show statistically normal distribution of protein, wet and dry gluten contents, and Zeleny sedimentation values (Figure 1). SPSS software was used for statistical analyses.

**Reference analysis**

Moisture, protein, and wet (WG) and dry gluten (DG) contents were determined according to the approved AACC methods (AACC 2000), and Zeleny sedimentation (ZS) was determined according to the approved ICC standards (ICC 1994). All parameters were determined on each flour sample in duplicate. The protein was reported on a dry matter basis (db), while wet gluten, dry gluten, and Zeleny sedimentation were reported on a 14% moisture basis (mb).

**NIRS**

Wheat flour samples were scanned on a NIRSystems 6500 scanning spectrophotometer (Foss NIRSystems Inc., USA) in reflectance mode. Spectra were recorded in log 1/*R* (*R*, reflected beam) format at 2 nm intervals from 1100-2500 nm. The analysis was carried out using small ring cup cells with a spinning sample cell holder. The spectrum of each wheat flour sample was obtained by collecting and averaging 32 individual spectral scans of the sample.

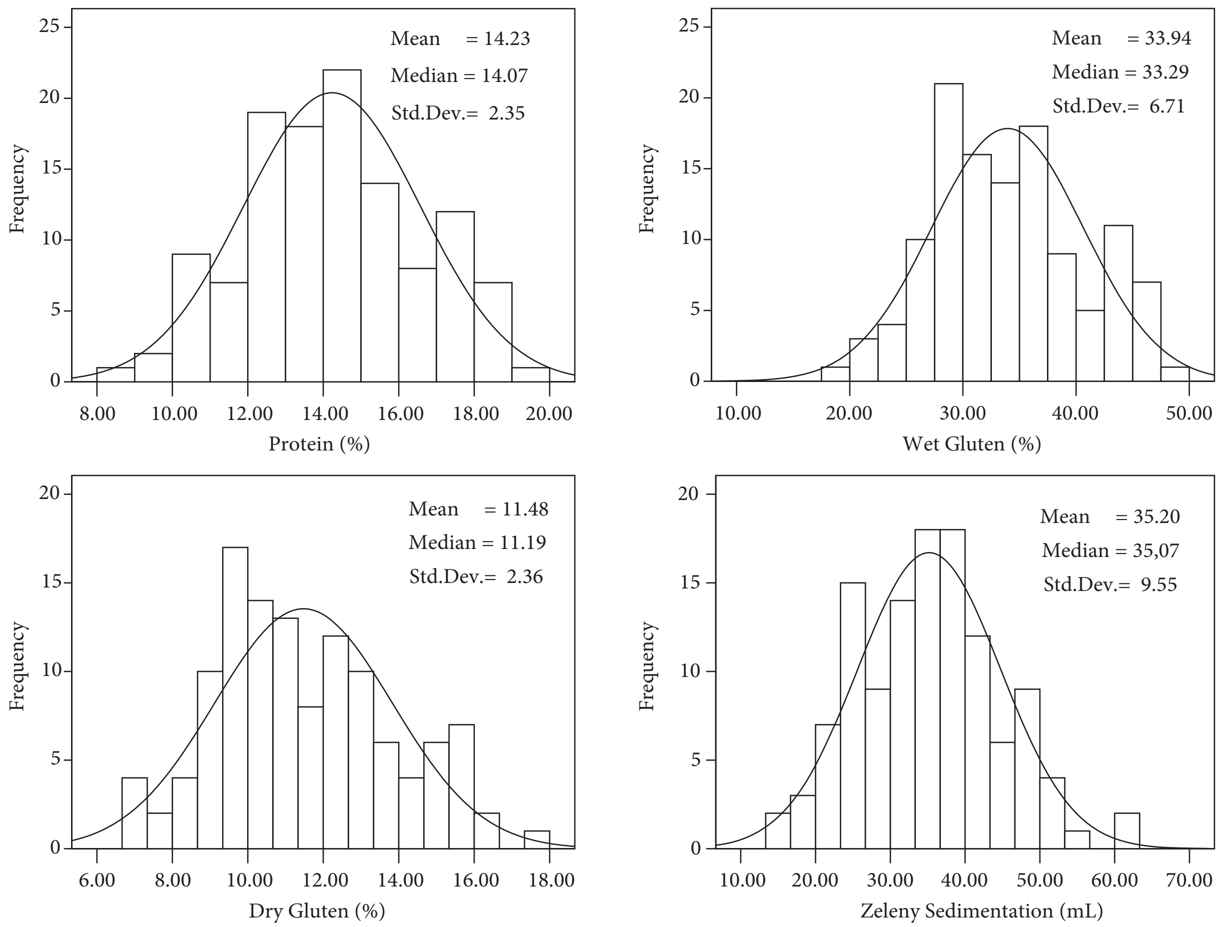


Figure 1. Characteristics of distribution for wheat flour samples.

**Calibration and validation**

Wheat flour samples were randomly divided by the software into separate calibration ($n$ = 80) and validation ($n$ = 40) sets, after sorting the original sample sets on the basis of each individual quality parameter. Samples with higher deviations were not left out. Multivariate regression was used to select wavelengths and relate *log 1/R* values to protein, wet gluten, dry gluten and Zeleny sedimentation from each set of spectra. NIRS calibration equations were developed using both modified partial least squares (PLS) regression and multiple linear regression (MLR) techniques. These regression techniques were applied to the *log 1/R* spectra, as well as the first and second derivatives of raw *log 1/R* spectra, and the prediction results obtained from each mathematical treatment were compared to select the best one. The statistics of most interest were the following: standard error of calibration (*SEC*), standard error of cross validation (*SCEV*), standard error of performance (*SEP*), and correlation coefficients ($r$) between values by reference and NIRS methods. These statistical parameters were used to select the optimum segment and gap values for calculating the derivatives, and were compared to evaluate which calibrations were most successful at determining protein, wet gluten, and dry gluten content in wheat flours.

## Results

Wheat flour samples were randomly selected for calibration by the software, and the remaining samples were used for the validation set. The statistical results of calibration and validation for the wheat flour parameters are summarized in Table 1. Strong correlations were obtained between NIRS predicted values and the reference data for protein, wet and dry gluten, and Zeleny sedimentation in both the calibration and validation sample sets. Log 1/*R*, first-derivative, and second-derivative mathematical treatments were applied successfully for each constituent of the wheat flours. The second derivative treatment gave the best $r$ values and lowest SEP and bias for protein, wet gluten, and dry gluten while the first derivative treatment gave the best results for Zeleny sedimentation. Calculating derivatives of spectra can remove scattering and other spectral differences that result in both slope differences and offsets among spectra (Yildiz et al. 2001).

The NIRS predicted values as compared with lab-measured values for protein, wet and dry gluten, and Zeleny sedimentation are shown in Figure 2. All parameters were predicted with sufficient accuracy.

## Discussion

Good results were obtained for determination of protein, wet gluten, and dry gluten indicating $r$ = 0.985, 0.976, and 0.953, respectively, while Zeleny sedimentation showed reasonable validation performance with $r$ = 0.924 (Figure 1). Miralbes (2003, 2004) reported that the prediction of protein, wet gluten, and dry gluten content of wheat flours by NIRS was straightforward and accurate. Similar

Table 1. Results of NIR calibration and validation sets for wheat flour characteristics.

| Constituent[a] | Calibration set ($n$ = 80) | | | Validation set (n = 40) | | | |
|---|---|---|---|---|---|---|---|
| | SEC[b] | SECV[c] | Multiple *R* | SEP[d] | Correlation (r) | Bias | Slope |
| Protein (%, db) | 0.347[e] | 0.032 | 0.990 | 0.377 | 0.985 | 0.028 | 1.024 |
| Wet gluten (%, 14% mb) | 1.938[e] | 1.242 | 0.961 | 1.36 | 0.976 | 0.276 | 1.026 |
| Dry gluten (%, 14% mb) | 0.716[e] | 0.165 | 0.958 | 0.635 | 0.953 | 0.066 | 0.936 |
| Zeleny sed. (ml, 14% mb) | 5.10[f] | - | 0.850 | 3.74 | 0.924 | -0.747 | 1.072 |

[a] db: dry matter basis, mb: moisture basis; [b] standard error of calibration; [c] standard error of cross validation; [d] standard error of prediction; [e] PLS model using 2nd derivative spectra; [f] MLR model using 1st derivative spectra.

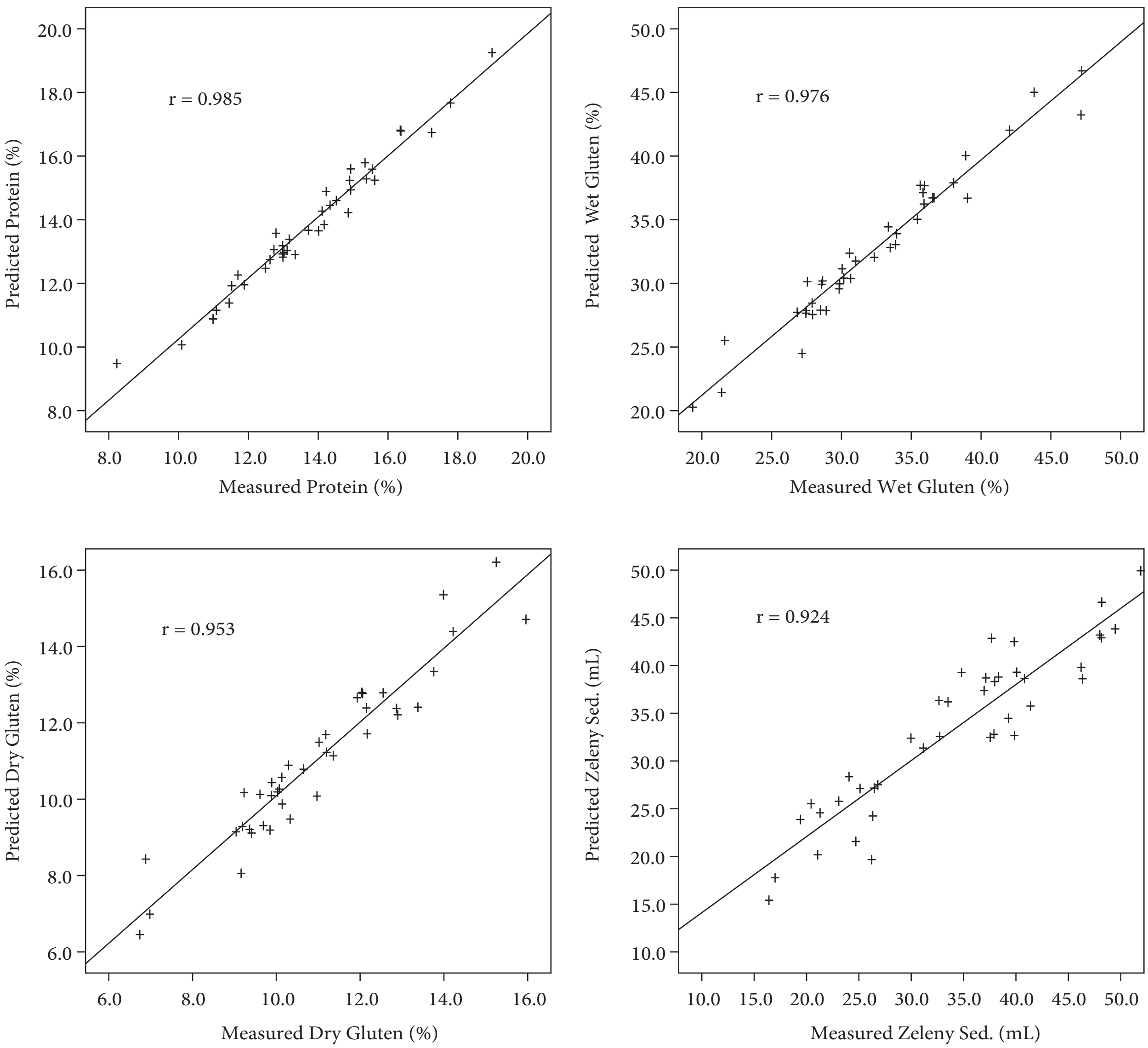


Figure 2. Scatter plots of analytically measured and predicted values for protein, wet and dry gluten contents, and Zeleny sedimentation in the validation set of wheat flours.

results for these constituents were also obtained in our study. Some interesting results on protein content (Hruskova et al. 2001, $r = 0.963$; Hruskova and Famera 2003, $r = 0.992$; Dowell et al. 2006, $r = 0.97$; Jirsa et al. 2007, $r = 0.991$), wet gluten (Pawlinsky and Williams 1998, $r = 0.86$; Miralbes 2004, $r = 0.97$; Ertugay et al. 2007, $r = 0.902$), dry gluten (Miralbes 2003, $r = 0.96$) and Zeleny sedimentation (Hruskova et al. 2001, $r = 0.548$; Hruskova and Famera 2003, $r = 0.833$; Jirsa et al. 2008, $r = 0.624$) were carried out by NIRS.

NIRS was used to develop calibration models for the prediction of protein, wet and dry gluten and Zeleny sedimentation. The Zeleny sedimentation value had a slightly lower accuracy than protein, wet gluten, and dry gluten constituents. It was concluded that NIRS was apparently capable of prediction of wet and dry gluten and Zeleny sedimentation. The good performance of the NIRS for wet and dry gluten appears to be strongly dependent on the correlation to protein content. Since NIRS results were better, it appears that NIRS is not strictly measuring protein content as a means for predicting gluten, especially for wet gluten.

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