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Use of Near Infrared Reflectance and Transmittance Coupled to Robust Calibration for the Evaluation of Nutritional Value in Naked Oats

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ABSTRACT: A rapid accurate and precise method for simultaneous determination of β-glucan and protein content in naked oat samples, based on the coupling of near-infrared spectroscopy and chemometrics, is presented. In particular, three different spectroscopic approaches [near infrared reflectance (NIR) and transmittance (NIT) on flour and NIT on whole grains] and various spectral pretreatments were considered. To account for the possibility of outlying samples, a robust version of the PLS algorithm (namely partial robust M-regression) was used. All the models resulted as accurate as the reference methods, reflectance spectroscopy being the technique providing the best outcomes. Variable reduction by inclusion of the most relevant predictors only (as evaluated by VIP scores) resulted in simpler and, in one case, more parsimonious models, without loss in accuracy.

KEYWORDS: near infrared spectroscopy, naked oat, robust multivariate calibration, chemometrics, β-glucan, protein, partial robust M-regression (PRM)

INTRODUCTION

The recent knowledge of the relation between some food components and human health status has triggered a growing interest among consumers in functional food designed with the aim to provide specific health benefits, by contributing to disease prevention.[1,2] Following this trend, oat (*Avena sativa*) is considered one of the most important grain cereals for human consumption. Indeed, oat products are important sources of dietary fiber, β-glucan, good nutritional value proteins, vitamins and other components, which are demonstrated to be beneficial for human health. Furthermore, the interest in oats for the production of functional foods is increasing, due to the growing evidence of the physiological effects and the positive impact of soluble fiber, i.e. β-glucan, on some of the risk factors responsible for cardiovascular diseases.[3,4] β-Glucan is a large, linear nonstarch polysaccharide ($\beta(1\rightarrow3)/(1\rightarrow4)$-D-glucose units) mainly localized in the endosperm cell wall of oat and barley.[5] The main property of water-soluble β-glucan is its propensity to produce highly viscous solutions;[6,7] this characteristic is linked to its potential health benefits.[8] On this basis, raw material that is more suitable for oat based food production is strongly requested. There are quite large differences in oat grain composition among varieties, particularly between husked and naked oats, the latter being nutritionally superior.[9,10] Naked oats, in which the hulls are not retained as in common oats,[11,12] usually contain 17 and 22% d.m. of protein and 3−6% d.m. of β-glucan, depending on genetic and agronomical factors.[13] However, the availability of naked oat germplasms in Europe is scarce. Accordingly, two new registered domestic varieties of naked oat "Irina" and "Luna" (2009) are being cultivated in Italy for their potential use for human nutrition. In this framework, maintaining a high level or even increasing protein and β-glucan contents are key objectives of oat breeders, in relation to the important role that these nutrients play in the human diet. Therefore, following the industry requirements of naked oats for human consumption, the selection of new lines with high nutritional potential requires accurate and rapid methods to evaluate the quality traits in the material produced in breeding programs. With respect to this, it must be pointed out that, in most of the cases, the traditional analytical methods are destructive and, particularly for β-glucan quantification,[14−16] require long determination times and high costs. On the other hand, in relation to its well-known advantages, near infrared spectroscopy can meet the breeding requirements[17,18] and facilitate selection based on traits of nutritional interest, such as protein and β-glucan content. Indeed, the potential of near infrared reflectance (NIR) spectroscopy to predict dietary fiber components has been already reported for other cereal products. For instance, previous works have shown the ability of NIR and near infrared transmittance (NIT) technologies to quantify soluble and insoluble dietary fiber in kernels.[19] Furthermore, other authors have shown the possibility of NIR spectroscopy to analyze the barley β-glucan content,[20−23] and, recently, Schmidt et al.[5] have compared different types of NIR instruments in the ability to measure β-glucan content in naked barley.

With the aim to assess the nutritive potential value in new naked oat genotypes during breeding work, this study focuses on the possibility of developing a rapid, accurate and precise alternative method for the simultaneous quantification of β-glucan and

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protein content in naked oat samples, based on the coupling of near-infrared spectroscopy and chemometrics. Additionally, as different spectroscopic approaches can be adopted when carrying out the measurements, the results of working in reflectance or in transmission and, in the latter case, of analyzing whole grains or milled samples are also evaluated and compared.

MATERIALS AND METHODS

Naked Oat Samples. The whole data set comprises 168 naked oat samples from 12 varieties, originally coming from Italy and other European countries and being representative of a large genetic range. The samples were obtained from multiyear trials carried out in Rome during three successive years (2007−2009) with the aim of evaluating the influence of different climatic conditions and some agronomic factors (nitrogen fertilizer and seeding rate) on the nutritional value of naked oat varieties.

Chemical Analyses. An aliquot of each sample was milled to a particle size of 0.5 mm using a Fritsch 14.702 laboratory mill (Fritsch GmbH, Markt Einersheim, Germany). Protein content was assessed by the Kjeldhal method.[24] β-Glucan concentration was measured according to the enzymatic method of McCleary and Codd[14] using a Megazyme β-glucan kit (Megazyme International Ireland Ltd., Bray, Ireland).

NIR and NIT Analysis. Transmission measurements of grain and flour were recorded on a grain analyzer (Infratec 1241; Foss Analytical AB, Höganäs, Sweden) between 800 and 1048 nm scanning at 2 nm interval yielding. The optical path length is 18 mm in whole grain samples. For the analysis of the flour samples 3 mm long cups were used. The collection of reflectance spectra was performed with a Foss NIR-system model 6500 (Foss Analytical AB, Höganäs, Sweden), which extends measurements into the visible range down to 400 to 2500 nm scanning at 2 nm intervals. The analyses were made on ground grain samples using a sample transport module.

Calibration Development. To relate the spectral measurements to the analyte (β-glucan or protein) content, calibration models were built using multivariate latent variable-based regression[25] and VIP[26] were used to further improve and interpret the model. As several instrumental effects can hinder or worsen the performances of the calibration models, different kinds of spectral pretreatments (MSC, detrending, first and second derivatives, and the combination of MSC with any of the latter three) were tested.[27] In the case of reflectance data, pretreatments were applied separately to the range 400−1098 nm and 1100−2498 nm to account for the possibility of different scatter effects. Moreover, to account for the possibility that outliers are present in the data set a robustified version of the PLS algorithm, namely, partial robust M-regression, was used. Partial robust M-regression[28] adopts an iteratively reweighted regression scheme to compute the latent variable model, where weights are introduced to take into account both vertical (in error terms) and horizontal (in leverage) outlyingness. As the readers may not be familiar with robust regression, a brief description of the partial robust M-regression (PRM) algorithm is provided in the following subsection.

Partial Robust M-Regression (PRM). Partial least squares (PLS) regression[25] is a well-known regression technique whose large success and wide use rely on its ability to deal with numerous and collinear x-variables and on the possibility of tuning the model complexity. These properties are especially useful when dealing with spectral data, where many and strongly correlated x-variables are recorded. However, standard PLS algorithms are known to be severely affected by the presence of outliers in the data or deviations from normality. Indeed, on one hand the resulting model may fit the anomalous observations (and mask their erroneous nature), while on the other hand, some good data points might be identified as outliers. To overcome these drawbacks, several robust alternatives to classical PLS have been proposed, with the aim of detecting data contamination and estimating a regression model that mainly fits the "good" data. Outliers can then be identified easily by their residuals from this robust fit. In this paper, among the different robust PLS regression strategies, the use of partial robust M-regression (PRM), introduced in 2005 by Serneels et al.,[28] was chosen.

In PRM, as in standard PLS, the original predictors are replaced with orthogonal latent variables with maximum covariance with $\boldsymbol{y}$. Therefore, the original regression problem is reduced to the latent variable model:

$$\mathbf{Y} = \mathbf{TB} \quad (1)$$

where $\mathbf{T}$ is the score matrix, made up of the coordinates of the samples in the latent variables space, and $\mathbf{B}$ are the regression coefficients relating the dependent variables $\mathbf{Y}$ to these new set of descriptors. The scores $\mathbf{T}$ are obtained from the original variables $\mathbf{X}$ via a weight matrix $\mathbf{W}$:

$$\mathbf{T} = \mathbf{XW} \quad (2)$$

When estimating the regression coefficients, two kinds of outliers can be influential: leverage points (horizontal outliers), which are observations far away from the majority of the data in the multivariate space of the predictors (not necessarily leading to large residuals), and vertical outliers, which may not be atypical in the regressor space but have large residuals. PRM offers good robustness properties as it deals with both kinds of outliers, adopting a downweighing scheme. Accordingly, for each observation $(\boldsymbol{x}_i, \boldsymbol{y}_i)$, two sets of real-valued weights in the range 0−1 are introduced: a weight w^x_i, which is responsible for dealing with leverage points, and a weight w^y_i, which is relevant for vertical outliers. In the modeling phase, these weights are iteratively adjusted, in order to diminish the negative influence of outlying objects on the regression model. Eventually, observations close to the center of the data cloud in the predictor space will receive a horizontal weight w^x_i close to 1 while for leverage points the value of this weight will be close to zero. Similarly, observations resulting in a high residual will receive a vertical weight w^y_i close to zero.

Software. Spectra were exported from the instrument by means of WinISI Project Manager v.1.50 software (Infrasoft International LLC, State College, PA). Pretreatment and data set splitting were carried out using in-house routines, while robust calibration was performed using the multivariate toolbox TOMCAT.[29] All routines were run under MATLAB environment (The Mathworks, Natick, MA).

RESULTS AND DISCUSSION

The measured spectra were used to build robust calibration models for β-glucan and protein content. In particular, in a first stage the performances of the different spectroscopic approaches (reflectance on flour, transmittance on flour and transmittance on whole grains) were evaluated independently. Successively, a comparison among these three approaches was carried out. In all cases, as far as the model building is concerned, since the two analytes are independent of one another, robust calibration was performed separately for each dependent variable.

NIR Analysis on Flour. NIR analysis was carried out on 166 naked oat flour samples, each one measured in duplicate, resulting in a total of 332 recorded spectra. For each of the 166 samples, β-glucan (range: 2.39−4.33% as is) and protein content (range: 12.48−21.53% as is) were also measured according to reference methods described in the previous section. As near-infrared data can suffer from the presence of scattering or other undesired signal contributions, in the modeling phase, different kinds of pretreatment (MSC, detrending, first and second derivatives, and the combination of MSC with any of the latter three) were tested for their effectiveness. In each case, after pretreatment, spectral data matrices were built by averaging the pretreated signals of the two replicated measurements for each

sample. The next step was then to divide the whole data set into training and test sets (the former to build the model, the latter to validate it). As we decided to use a robust algorithm (partial M-regression) to build reliable calibration models even in the presence of possible outliers, a procedure based on the Kennard−Stone algorithm[30] was used to split the data set: indeed, by including all the most diverse samples in the training set, the use of this algorithm guarantees that, in case outliers are present, they are all put in the calibration set. To account for the fact that different pretreatments had to be tested and that as much as possible of the variation after scatter or baseline removal was covered in the selection, at the same time having a unique sample splitting scheme to be able to compare the outcomes after the different pretreatments, the following procedure was adopted. The Kennard−Stone algorithm was applied on each of the data matrices corresponding to the different pretreatments using a splitting ratio of about 3:1 (120 samples in the training set and 46 in the test set). Then, for each sample the frequency of selection as part of the training set was computed and all the samples being selected more than 4 times were included in the final training set (118 samples), while all the remaining were left out as the test set (48 samples). A representation of the training/test splitting in the space spanned by the first two principal components is shown in Figure 1 for the different pretreatments. It should be stressed that, in general, the use of the Kennard−Stone algorithm for data set splitting has a potential drawback due to the fact that predictions on the test set may be too optimistic as the test samples are well positioned in the space spanned by the training ones. However, in the case of the splitting procedure used in this work, this drawback can be partly compensated by the fact that the final data splitting resulted from the combination of the outcomes of the selection on the differently pretreated data sets.

After splitting the data set according to the procedure described above, the training samples were used to build the robust calibration models for the quantification of β-glucan and protein content. As anticipated, partial M-regression was used to build robust latent variable-based calibration models. The use of this algorithm allows reliable results to be obtained also in the presence of both vertical and horizontal outliers in the training set. To ensure robustness of the algorithm, it is necessary to assume the largest possible percentage of data contamination, so in this study contamination level was set at 15%. With this setting, individual calibration models were built for protein and β-glucan content. In both cases, Monte Carlo cross-validation with 500 iteration and 30 objects left out per iteration was used to determine the optimal complexity of the models.

The results of the modeling phase are summarized in Table 1 (in all cases, the spectral pretreatments described above were followed by robust centering by subtraction of the L1-median).

It can be seen from Table 1 that, in the case of β-glucan, MSC was the pretreatment resulting in the best performances in cross-validation and therefore was the one selected to build the final model resulting in a root-mean-square error in calibration (RMSEC) of 0.177 and a root-mean-square error in cross-validation (RMSECV) of 0.198 (5 LVs were retained). The optimal model was then tested on the external validation set, resulting in a comparable error (root-mean-square error in prediction, RMSEP = 0.236, see Table 1). These results can be visualized in Figure 2a where the observed vs predicted graph is reported in the case of both training (median of the Monte Carlo cross-validated estimation) and test samples.

Inspection of the same Figure 2a can be useful to show the importance of using a robust calibration approach for model building. Indeed, for instance, one of the samples in the calibration set appeared to have a β-glucan content of 0% (most likely due to reference error): this is an example of vertical outlyingness. Together with this rather evident vertical outlier, the PCA plots in Figure 1 suggested that there could be other possible horizontal outliers in the data set. In this situation, the traditional approach would be to remove outliers from the training set (using some diagnostic tool) and then build a classic PLS model on the supposedly clean calibration data. On the other hand, PRM not only results in a reliable model even in the presence of severe outliers but provides also an a posteriori model-based

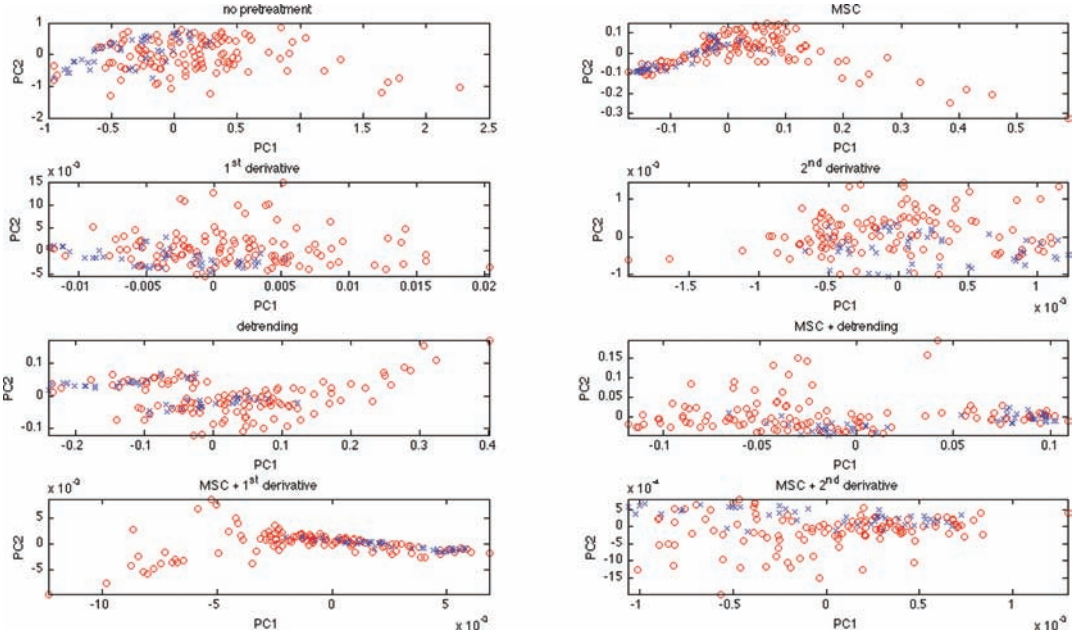

Figure 1. Representation of the training/test set splitting for the data set made up of NIR measurements on flour samples. Samples are projected onto the space spanned by the first two principal components, and the effect of the different pretreatments is shown [○ training set; × test set].

Table 1. Determination of β-Glucan and Protein Content. Characteristics of the Optimal PRM Models

	pretreatment	no. of LVs	RMSEC	RMSECV	RMSEP
		β-Glucan			
NIR (flour)	MSC + robust centering	5	0.177	0.198	0.236
NIT (flour)	MSC + 1st derivative + robust centering	1	0.181	0.208	0.246
NIT (grain)	1st derivative + robust centering	4	0.290	0.302	0.349
		Protein Content			
NIR (flour)	MSC + detrending+ robust centering	5	0.258	0.291	0.314
NIT (flour)	MSC + 1st derivative, robust centering	4	0.261	0.305	0.325
NIT (grain)	1st derivative, robust centering	5	0.271	0.287	0.336

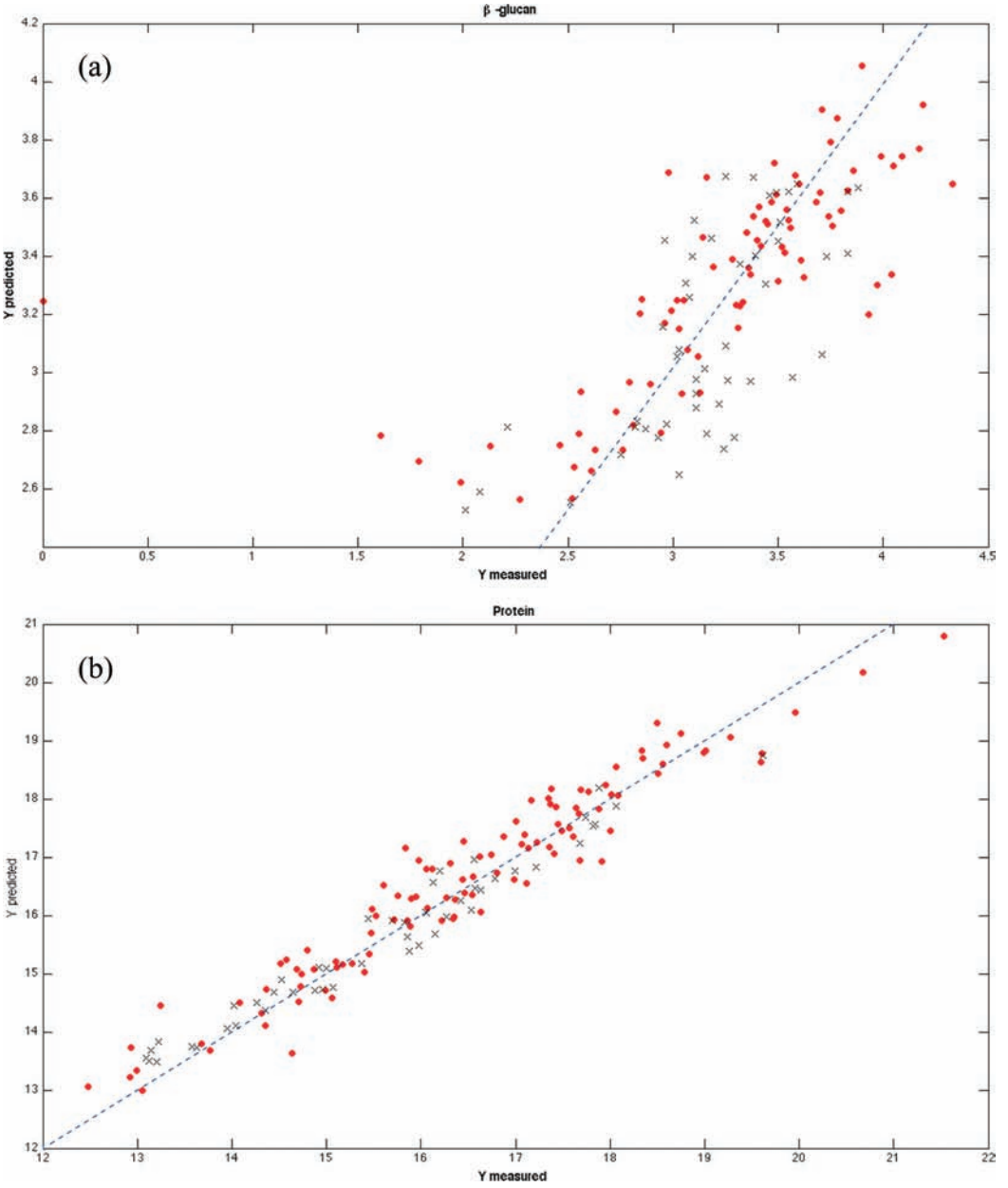

Figure 2. β-Glucan (a) and protein (b) determination on flour samples by NIR spectroscopy: observed vs predicted plot for the optimal calibration model [● cross-validated prediction; × test set].

estimate of the degree of outlyingness of each sample through the inspection of the weights. In particular, the plot of the residual vs leverage weights of the PRM model for the calibration of β-glucan is reported in Figure 3. It is evident from the figure that the sample for which a 0% β-glucan content was measured is clearly identified as an observation with a high degree of outlyingness. As expected, the main contribution to its outlyingness is given by the extremely high value of the residual, but it also shows a not negligible leverage. The same figure shows how other samples were significantly downweighed due to their extent of horizontal or vertical outlyingness.

As far as the calibration of protein content is concerned, MSC followed by detrending was the pretreatment resulting in the best performances in cross-validation and therefore was the one selected to build the final model resulting in a RMSEC of 0.258 and RMSECV of 0.291 (5 LVs were retained). The optimal model was then tested on the external validation set, resulting in a comparable error (RMSEP = 0.314, see Table 1). These results can be visualized in Figure 2b where the observed vs predicted graph is reported in the case of both training (median of the Monte Carlo cross-validated estimation) and test samples.

In order to check which spectral regions contributed the most to the calibration models, VIP values were computed for each wavelength:[26] VIP is an index of how much the single experimental variable contributes to the bilinear calibration model, and it is scaled in such a way that indices having VIP larger than 1 are considered to contribute significantly. In particular, the VIP scores for the optimal PRM models in the case of the analysis of NIR spectra of flour are reported in Figure 4. It can be seen from the figure that the regions contributing the most to the regression model for β-glucan are those between 400 and 700 nm, around 1150 nm and between 1900 and 2300 nm. The latter region is reported in the literature to be characterized, for polysaccharides, by −OH stretching/deformation and C−O/O−H stretching combination bands[31] and was identified by other authors,[5] as correlated with β-glucan content in barley samples. The significance of the region around 1900 nm can be hypothesized as a compensation for the contribution of moisture. On the other hand, the regions contributing the most to the bilinear model for the quantification of proteins are those between 400 and 700 nm, around 1100 and 1500 nm and between 2250 and 2498 nm: the latter two regions include wavelengths that can be ascribed to N−H deformation bands, and the inclusion of the lower frequencies can be interpreted as a way of compensating for the possible interference of starch or β-glucan itself.

Based on these considerations, in a successive attempt, calibration was repeated including in the data set only the wavelengths showing a VIP score larger than 1, to check whether this kind of variable selection could improve the modeling performances. Also with this reduced number of variables, the optimal complexity was 5 LVs in both cases as selected by Monte Carlo cross-validation (500 iterations leaving aside 30 samples per iteration) and the performances were comparable to those obtained on the full data set (RMSEC = 0.184, RMSECV = 0.202, RMSEP = 0.246 for β-glucan and RMSEC = 0.277, RMSECV = 0.305, RMSEP = 0.325 for proteins).

NIT Analysis on Flour. The same approach was then used to analyze the transmission spectra recorded on oat flour samples. In this case, only 54 samples (β-glucan range, 2.01−4.17% as is; protein range, 12.93−19.62% as is) were analyzed in duplicate resulting in 108 recorded spectra. Analogously to what already was described for reflectance analyses, different pretreatments were tested and, for each sample, replicate measurements were averaged after pretreatment. The same splitting procedure described in the previous section (based on the Kennard−Stone algorithm) was used to divide the 54 samples into training and test sets, so that 39 measurements were used to build the calibration models and the remaining 15 to externally validate it.

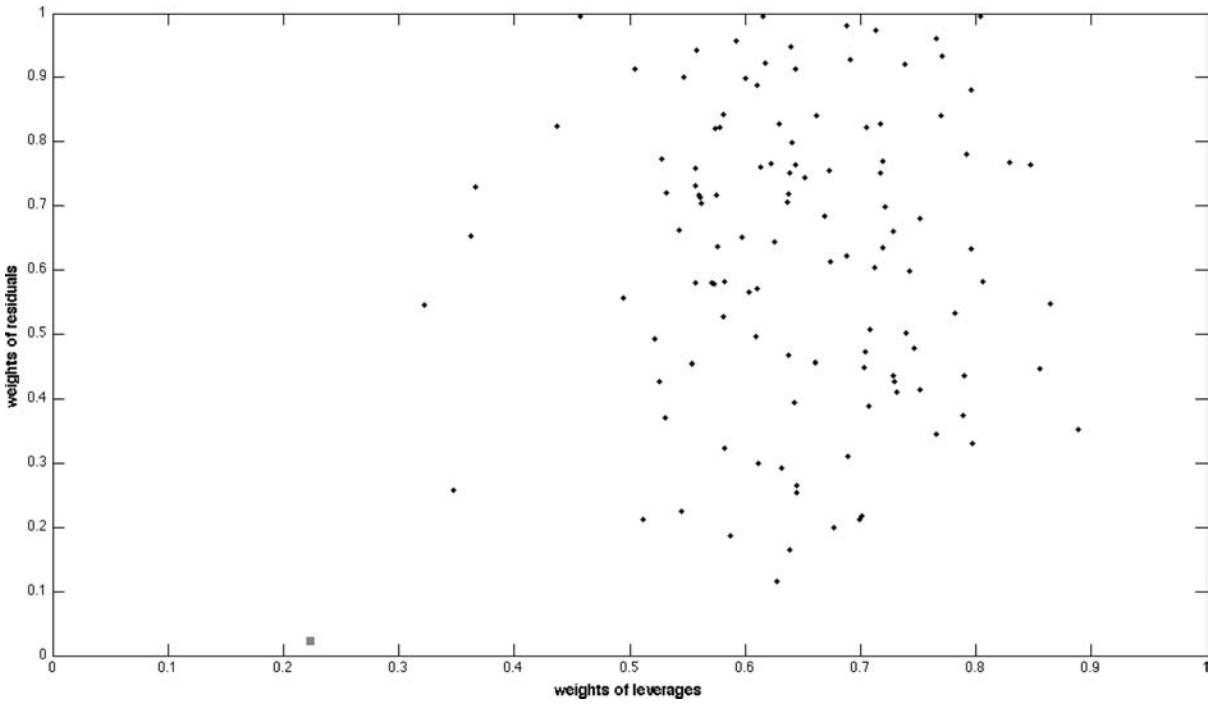

Figure 3. PRM modeling of β-glucan (NIR reflectance measurements on flour): leverage vs residual weights plot. The "evident" outlier having zero β-glucan as reference value is marked as a square.

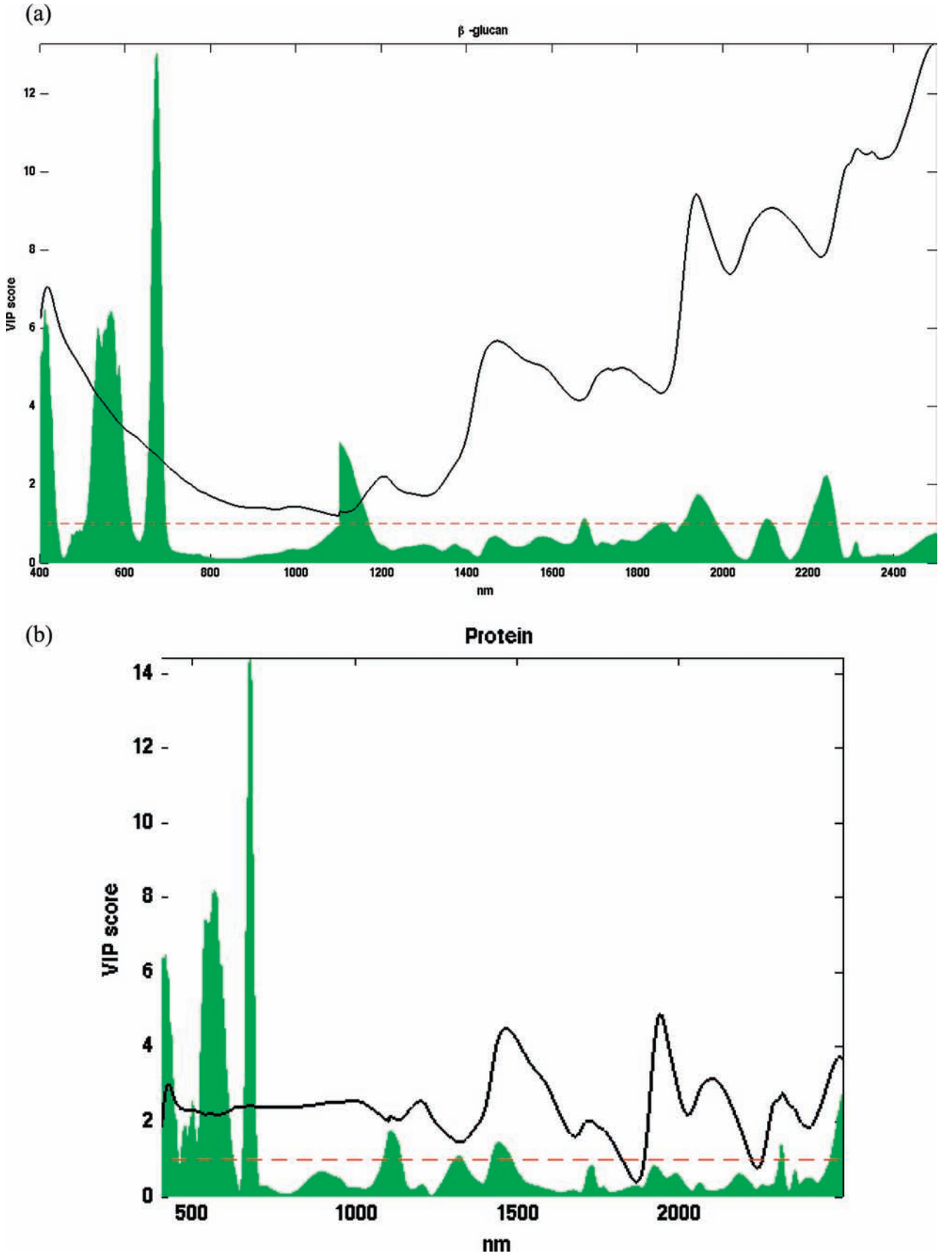

Figure 4. PRM modeling. VIP scores of the optimal PLS model for β-glucan (a) and protein (b) determination on flour samples by NIR spectroscopy are shown superimposed on the median pretreated spectra of the samples. Wavelengths having a VIP score higher than 1 are considered to contribute significantly to the model.

Monte Carlo cross-validation (500 iterations removing 10 samples a time from the training set) was used to select the optimal complexity of the PLS models and the best pretreatment for the spectral data. In particular, both for proteins and β-glucan, MSC followed by first derivative (Savitzky−Golay: 15 point window, third order polynomial) resulted to be the optimal preprocessing. Accordingly, the best PRM model for β-glucan included 1 LV and resulted in RMSEC = 0.181 and RMSECV = 0.208, while that for proteins included 4 LVs and resulted in RMSEC = 0.261 and RMSECV = 0.305 (Table 1). When applied to the 15 measurements left out to constitute the external validation set, very good performances were obtained (RMSEP = 0.246 and 0.325 for β-glucan and proteins, respectively). These results can also be visualized in Figure 5 where the observed vs predicted graph is reported in the case of both training (median of the Monte Carlo cross-validated estimation) and test samples.

Analysis of the VIP scores was used also in this case to identify the spectral regions contributing the most to the calibration model. As shown in Figure 6, most of the relevant contributions are from the wavelengths between 900 and 920 nm and between 960 and 1000 nm in the case of β-glucan, while for protein the highest VIP values correspond to the wavelengths between 930 and 960 nm.

Also in this case, calibration was repeated including in the data matrix only the wavelengths having a VIP score larger than 1 to

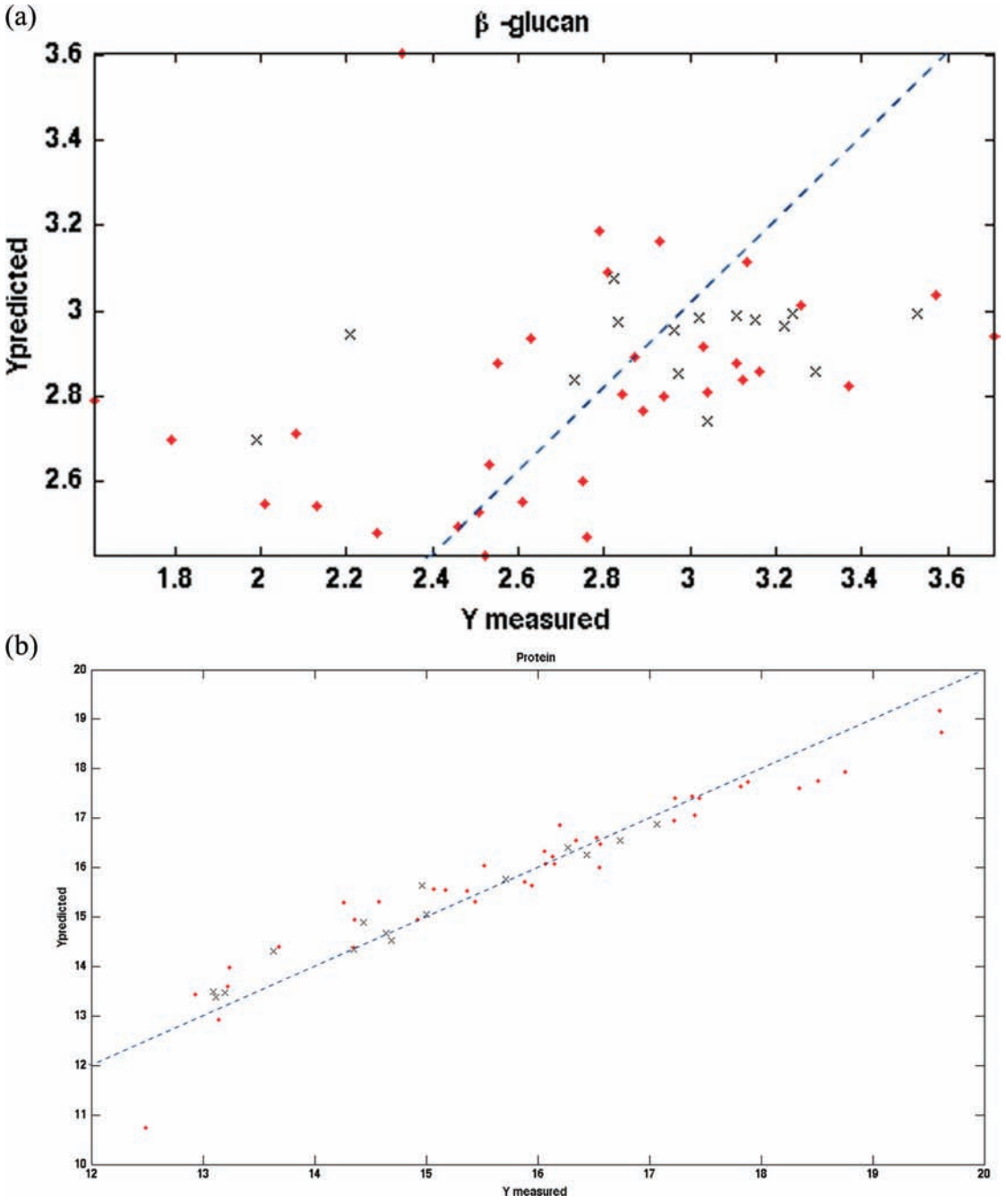

Figure 5. β-Glucan (a) and protein (b) determination on flour samples by NIT spectroscopy: observed vs predicted plot for the optimal calibration model [● cross-validated prediction; × test set].

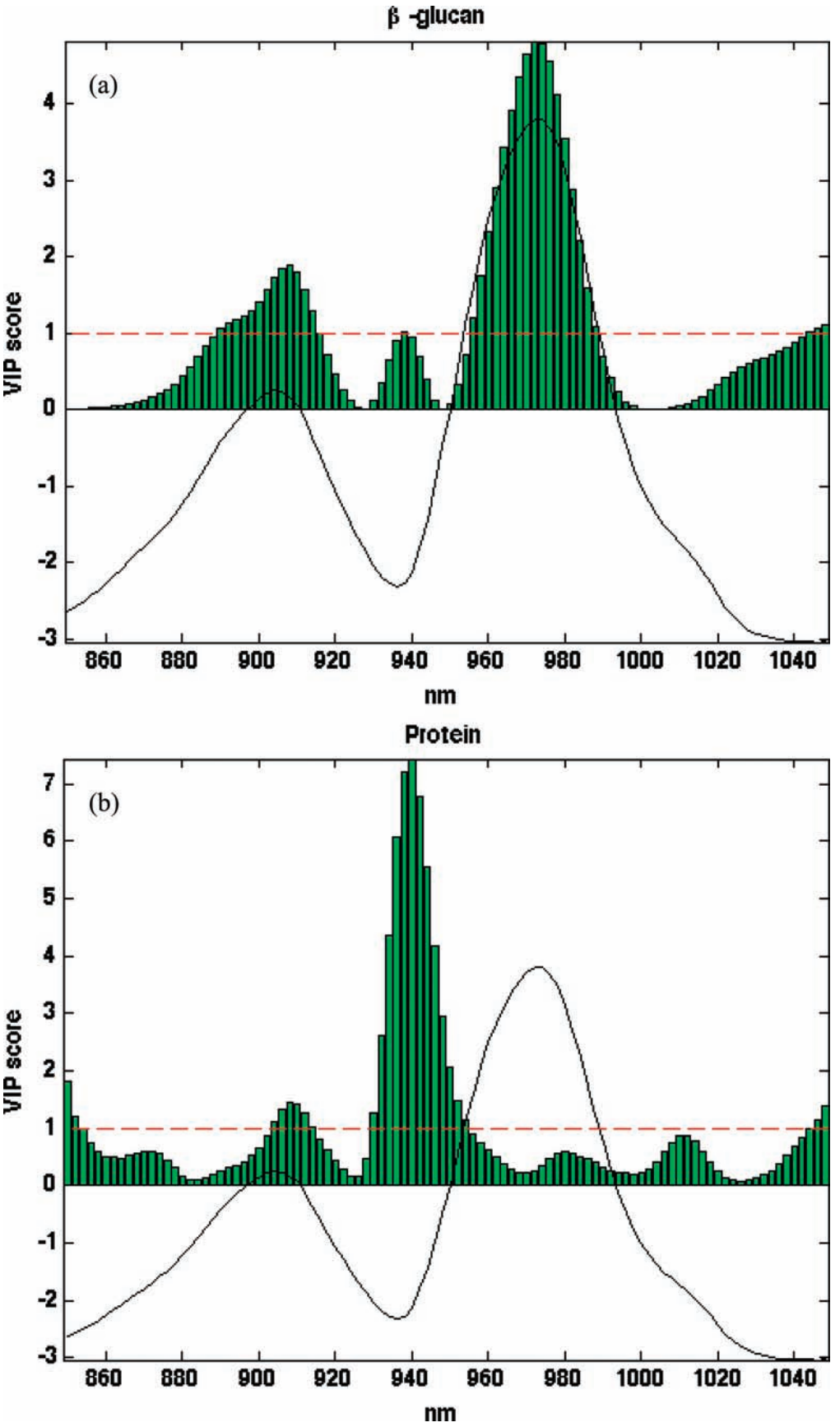

Figure 6. PRM modeling. VIP scores of the optimal PLS model for β-glucan (a) and protein (b) determination on flour samples by NIT spectroscopy are shown superimposed on the median pretreated spectra of the samples. Wavelengths having a VIP score higher than 1 are considered to contribute significantly to the model.

see whether variable reduction could improve the interpretability and/or the performances of the model. With this reduced number of variables, the optimal complexity was the same as for the whole wavelength range (1 LV for β-glucan and 4 LVs for proteins) as selected by Monte Carlo cross-validation (500 iterations leaving aside 10 samples per iteration) and the performances were comparable to those obtained on the full data set (RMSEC = 0.195, RMSECV = 0.221, RMSEP = 0.257 for β-glucan and RMSEC = 0.267, RMSECV = 0.319 RMSEP = 0.338 for proteins).

NIT Analysis on Whole Grains. Finally, data recorded on 168 whole grain samples by transmission spectroscopy were analyzed to build robust calibration models to predict β-glucan (range: 2.24−4.17% as is) and protein content (range: 12.48−20.01% as is). The data set was then divided into training and test sets following the same approach based on the Kennard−Stone algorithm already described in the previous paragraphs: 114 samples were chosen as training and the remaining 54 as test sets. As in the previous cases, replicate spectral measurements were averaged after pretreatment to build the predictor data matrices.

The optimal complexity and the best pretreatment among those examined in this study were chosen based on Monte Carlo cross-validation (500 iterations, 30 samples left out at each iteration). Both for the prediction of β-glucan and protein content, the best models were chosen to be the ones where first derivative (Savitzky−Golay: 15 point window, third order polynomial) was used to preprocess the data (Table 1). As far as β-glucan is concerned, 4 latent variables were retained in the final PRM model, which resulted in satisfactory modeling and cross-validation

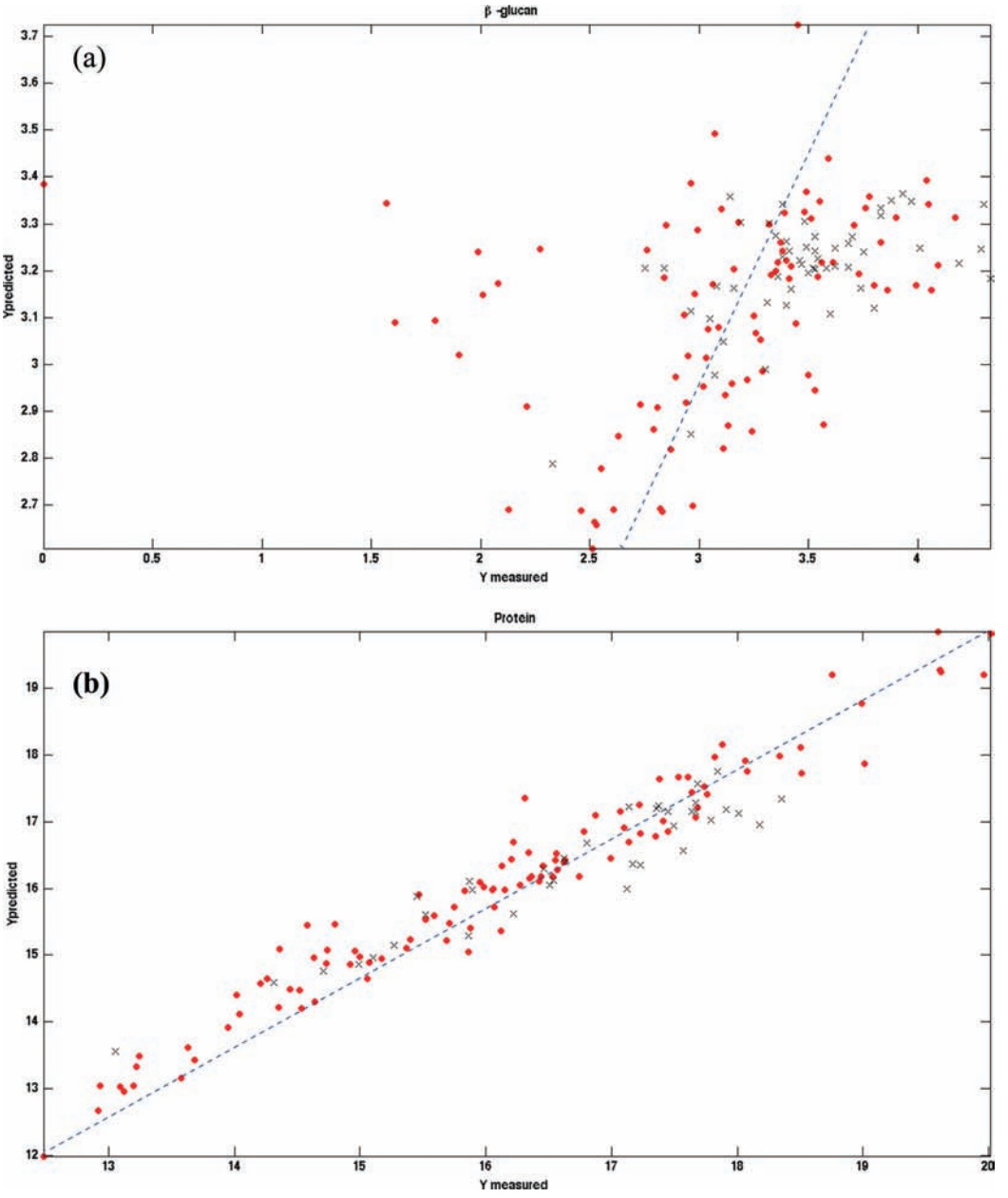

Figure 7. β-Glucan (a) and protein (b) determination on whole grain samples by NIT spectroscopy: observed vs predicted plot for the optimal calibration model [● cross-validated prediction; × test set].

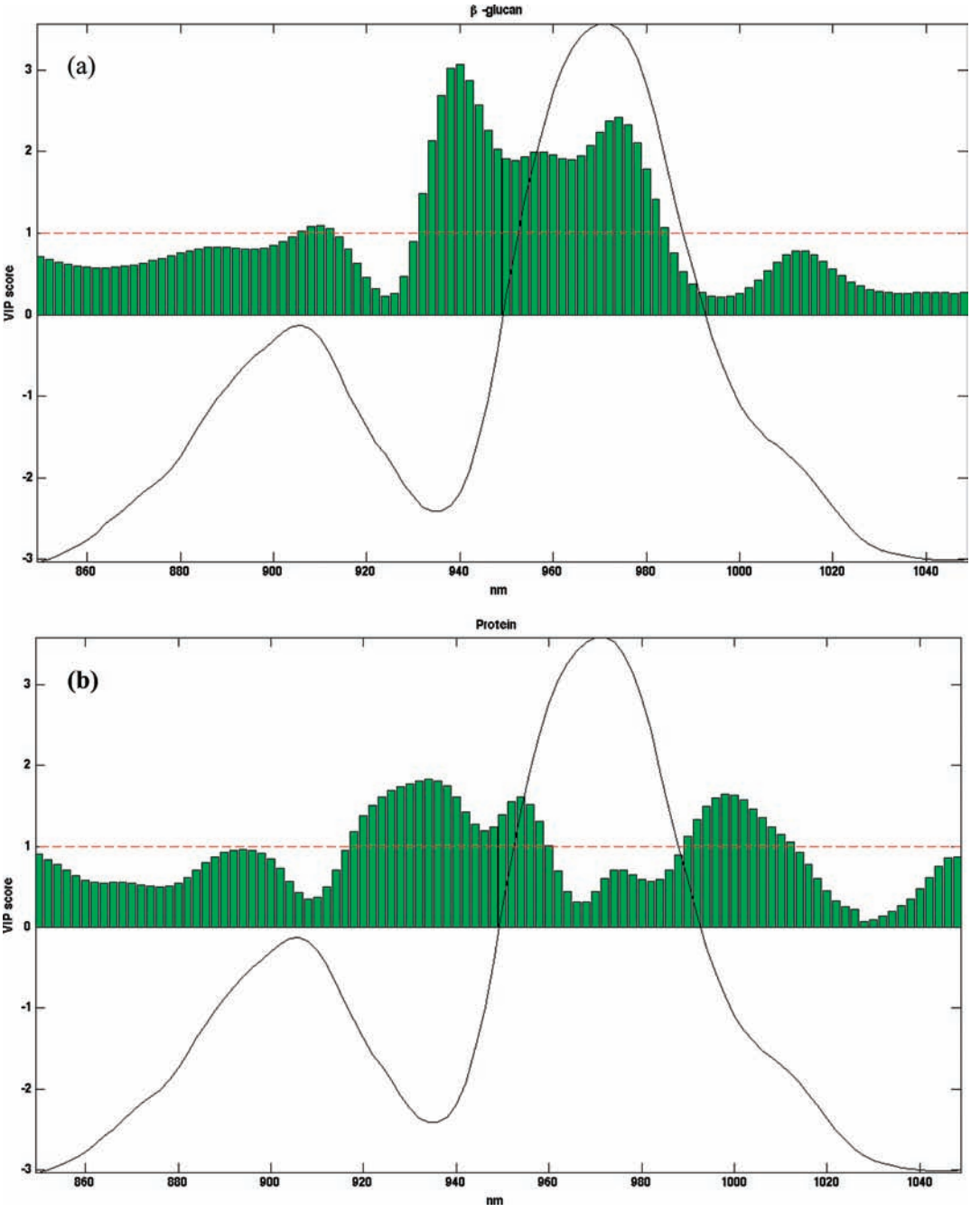

Figure 8. PRM modeling. VIP scores of the optimal PLS model for β-glucan (a) and protein (b) determination on whole grain samples by NIT spectroscopy are shown superimposed on the median pretreated spectra of the samples. Wavelengths having a VIP score higher than 1 are considered to contribute significantly to the model.

performances (RMSEC = 0.290, RMSECV = 0.302). When used to predict the β-glucan concentration of the test samples, a prediction error (RMSEP) of 0.349 was obtained. These values appear at a first glance higher than those obtained by the other examined spectroscopic approaches; however, as the test samples are different in the three cases, a direct comparison of the prediction errors is not straightforward and a different computational approach is needed (see next paragraph). On the other hand, as far as the determination of protein content is concerned, the optimal PRM model (5 latent variables) resulted in RMSE values comparable to those obtained with the other two approaches (RMSEC = 0.271, RMSECV = 0.287, RMSEP = 0.336). Calibration results can also be visualized in Figure 7, where the observed vs predicted plot is reported for the training (median of the Monte Carlo cross-validated estimation) and test sets.

Analysis of the VIP scores (reported in Figure 8) suggested that the spectral regions contributing the most to the bilinear models were those around 910 nm and between 940 and 1000 nm for β-glucan, while, for protein, those between 920 and 960 nm and between 1000 and 1020 nm. These selected regions are in good agreement with those identified in the case of NIT analysis of flour.

Table 2. Comparison between the Three Different Spectroscopic Approaches

	no. of LVs	RMSEC	RMSECV	RMSEP
		β-Glucan		
NIR (flour)	1	0.202	0.217	0.241
NIT (flour)	1	0.214	0.235	0.273
NIT (grain)	1	0.216	0.231	0.277
		Protein		
NIR (flour)	3	0.271	0.301	0.323
NIT (flour)	4	0.298	0.337	0.378
NIT (grain)	4	0.309	0.341	0.383

Analogously to what was done in the previous cases, calibration was repeated by including in the model only the wavelengths having VIP larger than 1. A slightly more parsimonious PRM model including 3 LVs only was obtained in the case of β-glucan, while 5 LVs were found to be the optimal complexity of the PRM model for protein content also on this reduced data set. For both analytes, the models computed on the variables selected using VIP scores gave performances with respect to those computed on the full data set (RMSEC = 0.299, RMSECV = 0.307, RMSEP = 0.357 for β-glucan and RMSEC = 0.267, RMSECV = 0.295, RMSEP = 0.343 for proteins).

Comparison between the Three Spectroscopic Methods. The results reported in the previous sections showed that both in the case of proteins and in the case of β-glucan the three spectroscopic approaches that were tested in this study gave all satisfactory results (the only exception being the analysis of β-glucan by NIT on grain samples resulting in higher RMSE values). However, as the number and kind of samples used in the training and in the validation phases were different in each of the three cases, a direct comparison of the three approaches based on the previous results is not feasible. Therefore, in order to compare among themselves the three tested approaches (reflectance measurements on flour, transmission measurements on flour and transmission measurements on whole grains) a further investigation was carried out by building a data set including only the samples that were analyzed with all three approaches for the sake of comparison.

This resulted in 53 samples (106 spectra) being selected for β-glucan and protein determination, respectively. To divide the samples between the sets, as the aim was to have the same training and test samples for each spectroscopic approach, data set splitting was performed applying a variant of the Kennard−Stone-based approach described previously. In this case, the Kennard−Stone algorithm was applied separately to the three data sets corresponding to the different spectroscopic approaches tested, each one after what appeared to be the optimal pretreatment (see Table 1). Then, for each analyte, samples being chosen as training set in at least two out of the three Kennard−Stone runs were selected as the final training set. Accordingly, 39 (β-glucan) or 38 (protein) samples were used to build the calibration models, which were then tested on the remaining (14 or 15, respectively). Here, as, at least in the case of reflectance measurements on flour, different pretreatments resulted to be optimal (Table 1), a different training/test splitting scheme was adopted for the two analytes. As in the previous stages of this study, Monte Carlo cross-validation was used to select the optimal complexity of the PRM models.

The results of this comparison are reported in Table 2 for both dependent variables.

It is evident from Table 2 that, when compared to one another, reflectance measurements on flour samples provide the better results in terms of predictive ability on unknown samples and this difference is slightly more evident in the case of the determination of β-glucan.

DISCUSSION

The results reported in the previous section allow some considerations and conclusions to be drawn. At first it was demonstrated that the use of near-infrared spectroscopy coupled to chemometric robust calibration techniques allows the simultaneous measurements of protein and β-glucan contents in naked oat grains with the accuracy requested for screening samples and significant advantages in terms of time and costs of the analyses. This can result in the possibility of speeding up the overall quality control of the samples and make specific breeding programs easier. Additionally, the use of chemometrics provides interpretable models: the analysis of the VIP scores can help the identification of the spectral regions that are more correlated with the analyte of interest. Besides interpretability, identification of the most important wavelengths in the spectra can result in a simpler and, in one case, more parsimonious model without any significant loss of the predictive ability. The results obtained in this work for β-glucan prediction were in agreement with the findings of Schmidt et al.[5] in naked barley by using analogous instruments. The calibrations included samples which covered the possible range of protein and β-glucan content, and the good accuracy both in cross-validation and in the external validation set revealed a close relationship between NIT−NIR and laboratory data.

When comparing among themselves the results obtained with the different spectroscopic approaches, it was found that in milled samples the NIR spectroscopy showed better performance in comparison with the NIT technology, probably because the limited wavelength range of the employed transmission spectrometer provided less available information.[19] Anyway, even if the results were comparatively worse, this study has also shown that NIT technology on whole grain has a sufficient accuracy to be used in naked oat quality control for fast and accurate analysis of nutritional components without requiring extensive sample preparation. This issue is of particular importance as many quality control laboratories working on cereals employ NIT spectrometers in a networked configuration: therefore, the possibility of obtaining accurate results using NIT allows the development of calibration models for quality control applications that can be easily transferred through the web from one instrument to another.[32]

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