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Near infrared reflectance spectroscopy (NIRS) to assess protein and lipid contents in *Avena sativa* L

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ABSTRACT - *Protein and lipid content are two of the main chemical traits of interest for genetic breeding of oat (*Avena sativa *L.). The purpose of this study was to determine whether NIRS could substitute conventional methods of determination of protein and lipid contents, and to verify any correlation of the two with each other and with physical grain traits. The estimates for protein and lipid content based on NIRS showed that the traits are selected more easily and quickly by this methodology. The low correlations between physical and chemical grain traits make the selection of inbred lines with large grains and high lipid levels possible. In the evaluated population the lipid and protein contents were not associated, i.e., it is possible to obtain genotypes with high levels of both traits.*

Key-words: breeding, grain quality, NIRS, oats.

INTRODUCTION

Genetic breeding has contributed considerably to the expansion of crops of great economic interest. Compared with the older American oat cultivars, the yields of the modern cultivars are 30-40% higher (White 2000). However, there is still room for improvement in terms of grain quality of this species. With higher protein and lipid contents underlying the chemical quality, the commercial value of this cereal may be enhanced. The oil content of oat caryopsis is the highest of all cereals; contents of over 18% were observed in genotypes obtained by recurrent selection (Humphreys et al. 1994). This fact demonstrates the potential of this chemical trait for breeding, particularly for the quality of oat oil, which is rich in monosaturated fatty acids (Peterson and Wood 1997) and widely recommended for health maintenance. Furthermore, oat is known for its antioxidant power, ascribed to the phenolic composites in the grain (Peterson 1992). Aside from the higher protein concentration, compared with other cereals oat is also outstanding in terms of the good balance of amino-acids in the caryopsis. The protein content varies from 12 to 24%, although changes in these contents hardly influence the proportion of amino-acids (Humphreys et al. 1994). Globulin is the predominant fraction and the lysine concentration is equal to that in rice (3 to 4%) and higher than in other cereals (Peterson 2000).

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The protein and lipid contents, together with the other chemical traits such as fibers and minerals, are fundamental from the nutritional point of view. Usually, the oat grain quality is determined based on physical traits. Many of these physical traits are nowadays evaluated by digital images (such as grain length and width), which are positively correlated with the industrial yield of this cereal. This method is being preferred since chemical analyses of genotypes under selection in breeding programs are hampered by aspects such as the time and cost required to prepare and analyze the samples. In this context, NIRS (Near-Infrared Reflectance Spectroscopy) can obtain accurate estimates more quickly, which facilitates and speeds up selection for traits of nutritional interest, such as lipid and protein contents. For this purpose, calibration curves consistent with the traits of the Brazilian cultivars are needed. The existing curves of American oat cultivars may produce misleading results, owing to the large difference between the cultivation conditions (climate, soil and management) of the respective regions.

The objectives of this study were to determine whether NIRS can substitute conventional methods of determination of protein and lipid content, and to verify if oat protein and lipid contents are correlated with each other and with physical grain traits.

MATERIAL AND METHODS

The population that originated the lines was obtained from the genotypes UFRGS 7 and UFRGS 17. The parents of UFRGS 7 were X1205 / FLA 1093, with an early cycle, short plant height, weight of a thousand seeds of 24g and leaf rust-susceptible. Oat cultivar UFRGS 17 released ten years after UFRGS 7, derived from the parents COR^2/CTZ^3/Pendek/ ME1563//76-29/76-23/75-28/CI833, with early cycle, tall plant height, weight of a thousand seeds of 33g is considered moderately susceptible to leaf rust, the main crop disease. UFRGS7 (small grains) with UFGRS 17 (large grains) were crossed aiming at an improved grain quality.

Ninety-nine F_7 lines obtained from the cited cross were used, as well as the parents. These genotypes were grown in the winter of 2001, at the Experimental station of the Universidade Federal do Rio Grande do Sul - UFRGS in Eldorado do Sul. Grain samples of lines and parents were milled and the grains selected by hand, to eliminate any broken and partially dehulled grains. Each sample was divided in two parts; one was ground for the chemical analyses and the other maintained as whole grains, which were used for the NIRS and digital image analyses, to determine hectoliter weight and the grain/hull ratio. The chemical analyses were carried out in a laboratory of the Departamento de Zootecnia da UFRGS, and the NIRS analyses in a laboratory of the Centro de Pesquisas em Alimentos – CEPA of the Universidade de Passo Fundo. The samples were dried and maintained at a moisture content of around 12%.

For the chemical analyses, a part of the milled samples was ground in a Knifetec 1095 Sample Mill (Foss-Tecator) for 50 seconds (granulometry 0.35 to 0.5mm). After milling, the sample was divided in two parts – one was used for protein content analysis by the micro-Kjeldahl method (AOAC 1995), and the other to determine the lipid content by ethereal extraction (AOAC 1995). Two replications were used for each analysis. The NIRS curves were calibrated based on the laboratory data of protein and lipid contents.

The sample spectra of whole and milled grains were obtained in a NIRSystems 5000 model. Approximately five grams of each sample were used for spectra reading. The spectra were stored as log (1/R) and processed by New Infrasoft International Software. Ninety-four lines and the respective parents were used to establish the calibration curves, one with data of whole and the other of milled grains. The standard error of calibration (EPC) is the difference between the real values of the sample constituents and those predicted by NIRS. R^2 represents the multiple coefficient of determination of the NIRS versus the sample reference value, used for the equation of calibration (R^2) and for the validation (R^2V). The results obtained based on the calibration using whole grains were correlated with the physical grain traits.

For the digital images, 50 whole dried grains were used, one of each of the lines and the respective parents. The following grain traits of these genotypes were determined by digital image analysis: area (mm^2), length (mm), width (mm), perimeter (mm) and grain shape factor (ratio of the minimum by the maximum diameter) with values between 0 and 1 (the closer to 1, the rounder is the grain). The pictures were transferred to the computer by an external video disk and the images analyzed in sequence by the program SIGMA SCAN / IMAGE 1.2. A standard size sheet of paper was used for the data conversion from pixels to millimeters.

The Pearson correlation coefficients were estimated for the F_7 generations between the chemical and physical grain traits, using the statistical program SAS (version 8.2)

RESULTS AND DISCUSSION

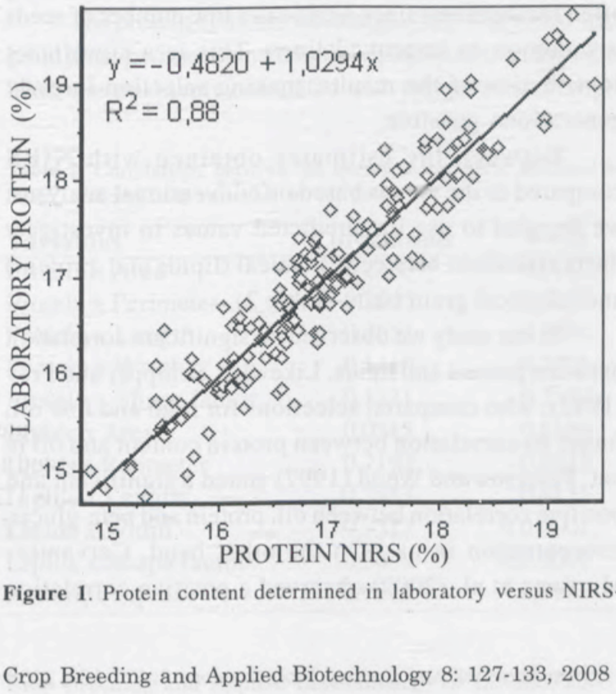
The low EPC values indicate low errors in the NIRS estimates. The protein as well as lipid contents were therefore calibrated with satisfactory accuracy (Table 1). Besides, the values of the multiple coefficient of determination (R^2) for the two traits were higher than 80%, for the curves representing the whole as well as milled grains, evidencing the accuracy of the estimated protein and lipid contents (Figures 1 and 2, respectively). According to Windhan et al. (1989), the best equation developed for the variable resulted from a combination of the low EPC values, of the EPVC with the R^2 produced in the cross calibration and validation stages.

Proteins as well as lipids were best adjusted when using whole grains - this raised a strong expectation for the use of NIRS in routine analyses or breeding programs. These results are highly relevant, since the conventional trait analyses, in some cases slow and vulnerable to a wide range of factors could be replaced by a far easier procedure. The result obtained with whole grains is very promising since a series of steps in the analysis of chemical grain quality traits is eliminated. The sample preparation is relevant since the particle size may induce changes in the reflectance (Borges et al. 2001). The use of whole grains excludes factors such as milling, size and particle uniformity, which is favorable for more accurate results.

Factors such sub-sampling, particle size and

Table 1. Number of samples (N), mean of the samples, standard error of calibration (EPC), coefficient of determination (R^2), standard error of cross validation (EPVC) and coefficient of determination of validation (R^2V) for protein and lipids in whole and milled oat grains

Grains	Variables			Validation statistics			
		N	Mean	EPC	R^2	EPVC	R^2V
Milled	Protein	156	16.80	0.36	0.89	0.38	0.88
	Lipids	122	5.87	0.25	0.90	0.27	0.88
Whole	Protein	175	16.98	0.32	0.91	0.34	0.89
	Lipids	175	5.79	0.33	0.83	0.35	0.81



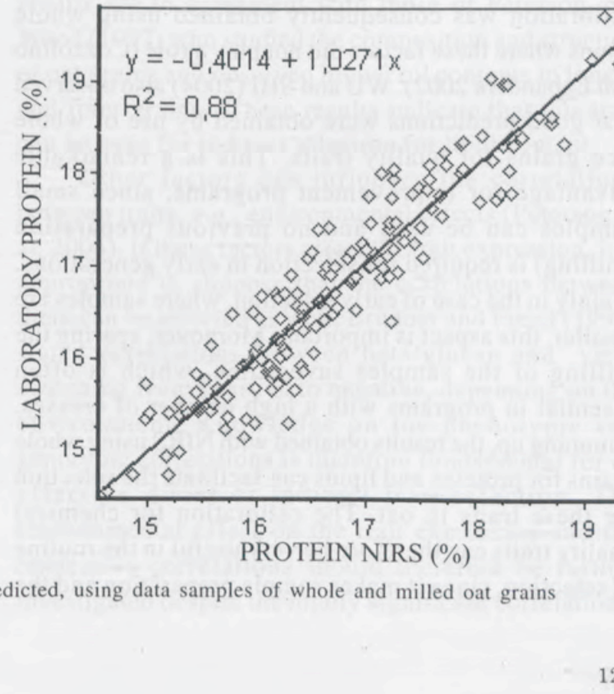


Figure 1. Protein content determined in laboratory versus NIRS-predicted, using data samples of whole and milled oat grains

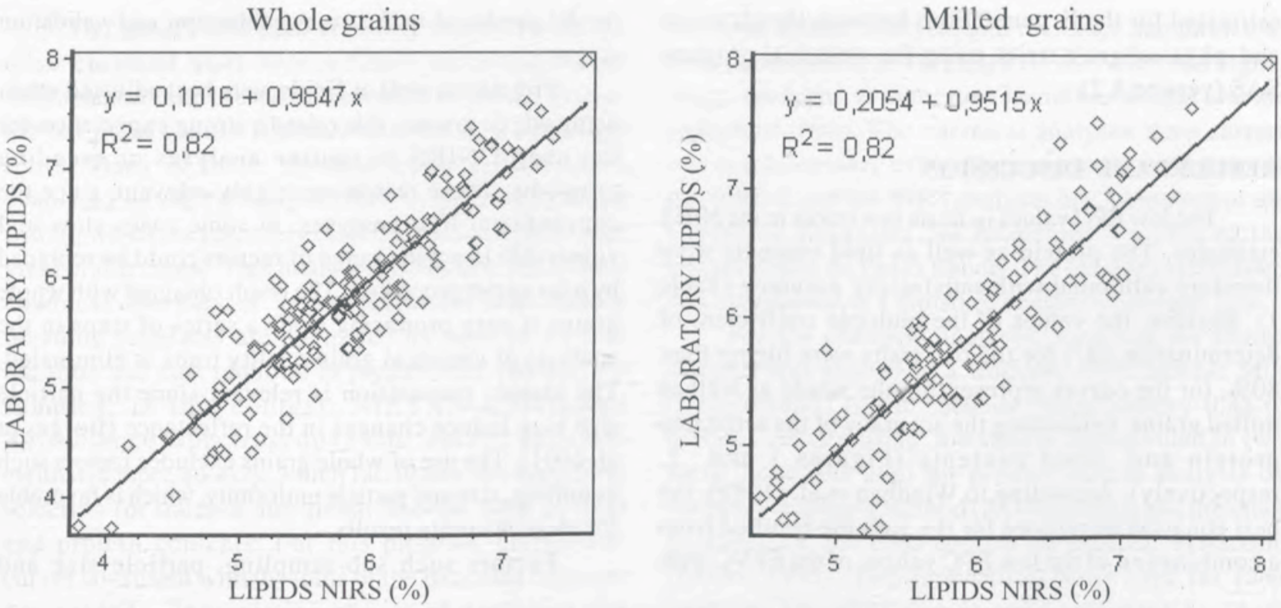


Figure 2. Lipid content determined in laboratory versus NIRS predicted, using data samples of whole and milled oat grains

residual moisture in the sample surface can affect the performance of the calibration since the radiation distribution depends on the physical traits of the sample (Borges et al. 2001). Variations in the particle size explain up to 90% of the variance in the NIRS spectrum, influencing the predicted values. An optical effect due to moisture in the cell surface can cause some degree of interference. Variations in the particle size may have interfered with the establishment of the curves obtained here, when using milled grains. The most reliable calibration was consequently obtained using whole grains where these factors did not play a role (Cozzolino and Labandera 2002). WU and SHI (2004) also observed that good predictions were obtained by use of whole rice grains for quality traits. This is a remarkable advantage for improvement programs, since small samples can be used and no previous preparation (milling) is required for selection in early generations. Mainly in the case of early selection, where samples are smaller, this aspect is important. Moreover, sparing the milling of the samples saves time, which is often essential in programs with a high number of crosses. Summing up, the results obtained with NIRS using whole grains for proteins and lipids can facilitate the selection for these traits in oat. The calibration for chemical quality traits can therefore be very useful in the routine of selection, since it makes sample preparation and the conventional chemical analyses in the laboratory unnecessary, but mainly for using non-destructive sample, that is, the seed that represents the sample with desirable traits can be used in the following generation of the breeding program.

The speed in the analyses and no need of expenses with chemical products are doubtlessly the main advantages of the NIRS method. The importance of this study with calibration for whole grains lies in the fact that whole grains can be used again. This is often fundamental since a critically low number of seeds is common in improved lines. This is a significant contribution of the results, making selection in early generations possible.

Between the estimates obtained with NIRS compared to the values based on conventional analyses, we decided to use the predicted values to investigate the correlations between chemical (lipids and protein) and physical grain traits (Table 2).

In our study we observed no significant correlation between protein and lipids. Likewise, Schipper and Frey (1992), who compared selections for high and low oil, found no correlation between protein content and oil in oat. Peterson and Wood (1997) stated a significant and positive correlation between oil, protein and beta-glucan concentration in oat. On the other hand, Cervantes-Martinez et al. (2002) observed a positive correlation between beta-glucan and protein, but a negative correlation of these with oil content. One should bear in mind, however, that in spite of the influence of the genetic germplasm constitution of each study on the correlations, environmental interactions are the reason for different results obtained by different authors and must be taken into consideration accordingly. Other reports point out that quality traits can also affect important agronomic traits, such as caryopsis yield and weight (Doehlert et al. 2001).

When the correlations between two traits of interest are negative, the selection of one of the traits affects the positive selection of the other. Cultivars with high nutritional quality but low yields can be cited as example, which are not readily accepted on the market. This is maybe one of the limitations of breeding for protein content in many crops, although some headway has been made. Such correlations are often caused by gene linkage or pleiotropy effects. In these cases trait breeding is hampered, so the linkages must be broken up by multiple crosses or induced mutation to make the breeding of genotypes with higher protein and lipid contents possible.

The correlations between chemical and physical parameters, despite significant, are low. This is evidenced by the correlation between shape factor and lipid content, which was the highest (56%). Nevertheless, the low correlations indicate that it is possible to combine the traits such as to obtain oat grains with the desired shape and chemical quality.

The protein contents were negatively correlated with grain area, perimeter and length. Lipid content on the other hand was positively correlated with perimeter and length and negatively correlated with shape (Table 2). Longer and fine grains tend to have a higher lipid percentage than rounder and shorter grains (Peterson 2000). The positive correlations between length and lipids and negative between width and lipids confirm this tendency, although the values of the correlations were low.

Table 2. Correlations between the chemical variables, obtained by NIRS, and physical variables of oat grains

Variables	Correlation	Prob
Protein x Area	-0.2580	0.0112
Protein x Perimeter	-0.2555	0.0120
Protein x Length	-0.2651	0.0090
Protein x Width	-0.1410	0.1706
Protein x Shape factor	0.1221	0.2359
Lipids x Area	0.0515	0.6184
Lipids x Perimeter	0.2197	0.0315
Lipids x Length	0.2553	0.0121
Lipids x Width	-0.3317	<0.0001
Lipids x Shape factor	-0.5609	<0.0001
Protein x Lipids	0.0872	0.3981

An important aspect of the industrial processing of oat is the resistance to breaking during the processing, mainly for the production of whole and roasted oat or oat flakes. Some studies demonstrated that longer grains break less during milling and attain a higher industrial yield; finer and longer grains are therefore preferable to reduce breaking. On the other hand, high lipid contents can be the cause of rancid deterioration and are undesirable in processed food. Cervantes-Martinez et al. (2002) found positive correlations between protein and beta-glucan content and a negative between beta-glucan and oil content. Cultivars can be developed specifically for milling, for example, as oatmeal, with a higher protein and beta-glucan content and low oil content, contributing to a fiber-rich and low energy diet. Thus, grain shape factors can be used in the indirect selection for chemical grain traits in oat, according to the final aim of the genotypes for oatmeal or whole grain.

The values for grain shape factor were lowest, i.e., the grains were longer, in genotypes with the highest lipid contents of the study population. These results are in agreement with those of Peterson and Wood (1997) who studied the composition and structure of oat grains and observed higher oil contents in longer and finer grains. These results indicate that this trait can be used for indirect selection for lipid content.

Other factors can influence the correlations between traits, e.g., environmental effects (Peterson et al. 2005). If these factors affect the trait expression, it is appropriate to suppose that the correlations between them can be affected as well. Brunner and Freed (1994) stated correlations between beta-glucan and yield switching from positive to negative, depending on the environment. Knowledge on the phenotypic and genotypic correlations is therefore fundamental for an effective direct or indirect trait selection. The environmental effect on the trait expression and the respective correlations should therefore be further investigated despite the highly significant correlations.

The knowledge on the correlations between traits of interest is indispensable for genetic breeding, since under direct or indirect selection they can cause favorable or unfavorable changes in important agronomic traits. The values of the correlations found here indicate that these can be explored in the sense of breeding for more than one trait of interest simultaneously. Doubtlessly, follow-up studies in more environments and for a greater number of important agronomic traits will be highly useful for a more in-depth understanding of the relations between these traits and will allow higher selection gains.

CONCLUSIONS

The estimates for lipid content and proteins obtained by NIRS allow the conclusion that this technique is effective and can facilitate the selection of these traits.

In these crosses lipids and proteins are not associated, which indicates that selection for high contents of these constituents in the same genotype is possible.

The low correlations between chemical and physical grain traits make the development of improved oat genotypes with large-sized grains and high lipid contents possible.

Uso da espectroscopia de reflactância no infravermelho próximo (NIRS) para análise do conteúdo de proteína e lipídios em *Avena sativa* L.

RESUMO - *Proteína e conteúdo de lipídios estão entre as principais características químicas de interesse para o melhoramento genético de aveia (*Avena sativa *L.). Os objetivos deste trabalho foram determinar se o NIRS poderia substituir métodos convencionais de determinação de conteúdo de proteína e lipídios, e verificar se proteína e lipídios estão correlacionados entre si e com características físicas dos grãos. As estimativas para conteúdo de lipídios e proteínas obtidas através do NIRS, demostram que esta metodologia é mais fácil e rápida para selecionar estes caracteres. Baixas correlações entre caracteres químicos e físicos de grãos tornam possível a seleção de linhagens com grãos grandes e níveis elevados de lipídios. Na população analisada, lipídios e proteínas não estão associados, o que torna possível obter genótipos com altos níveis para ambos caracteres.*

Palavras-chave: aveia, melhoramento, qualidade do grão, NIRS

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