Near infrared applications in the development of genetically altered grains

Daniel J. Dyer and Ping Feng
DuPont Quality Grains, Des Moines, Iowa 50322, USA.

Introduction

Agricultural biotechnology has entered the commercialization phase and is beginning to fulfill some of the promises of the technology. Much of the biotechnological revolution in agriculture will focus on the improvement in the functional utility of the harvested products. DuPont Quality Grains is a biotechnology-based business which utilizes genetic engineering and advanced breeding technologies to develop corn and soybean varieties which produce grain products having superior value in use in food products, animal feeds or industrial applications.

Whether the novel trait is introduced into the crop from exotic germplasm lines, through induced mutation, or through genetic engineering; the integration of the new trait into commercially usable varieties requires tracking the trait through plant breeding populations and analyzing thousands of segregating individuals to identify those bearing the desired trait. Supporting these types of biotechnology programs on a commercial scale requires analytical techniques which have high throughput, low cost, are safe, precise and preferably non-destructive. Near infrared (NIR) spectrometry is the first choice of analytical technique for many of these applications.

Among NIR techniques, both reflectance and transmittance spectrometry have valuable roles. When usable, transmittance of whole grain has the advantages of being faster and non-destructive. However, for less abundant or more challenging analytes, the higher sensitivity of reflectance spectrometry at longer wavelengths is necessary. Frequently, the decision between the use of reflectance and transmittance depends on whether the objective is to sort and rank order the individuals in a population, or whether accurate quantification is necessary. In many cases, both techniques used in series is most appropriate.

Results and discussion

In the DuPont Quality Grains business, we have utilized NIR spectrometry in the analysis of oil, protein and carbohydrate fractions of corn and soybeans, using either single seed or bulk samples. During the past seven years calibrations have been developed for oil and protein in individual corn kernels; oil, protein, starch, moisture, gross energy and oleic acid in whole bulk corn grain samples; and all of these plus individual specific amino acids in ground corn samples. For soybeans, calibrations have been developed for protein, oil and oleic acid in whole individual and bulk seed and these, plus stearic acid, lysine and stachyose, in ground samples. Table 1 provides some statistical description for the calibrations developed to-date.

All of the above calibrations for NIR transmittance have been developed for the Infratec model 1225 or 1221 analyzer. Those for NIR reflectance have been developed for the NIRSystems model 6500.

Table 1. Characteristics of NIR calibrations developed to-date.

Crop	Analyte	Range (%)	NIR transmittance *SEP*	NIR reflectance *SEP*
Corn	Oil	3–20	0.3	
	Protein	6–16	0.4	
	Starch	50–75	1.2	
	Moisture	6–32	0.7	
	Lysine	0.24–0.62		0.017
	Methionine	0.15–0.33		0.012
	Cysteine	0.19–0.39		0.013
	Threonine	0.3–0.6		0.013
Soybeans	Oil	14–23	0.40	
	Protein	37–55	0.65	
	Oleic Acid	15–60	3.6	2.2
	Stearic Acid	3–25		1.8
	Lysine	2.45–4.94		0.17
	Stachyose	0–5.2		0.44

In many cases, near infrared spectrometry is employed as a semi-quantitative technique through which samples can be rank-ordered for the purpose of selecting the desired individuals to progress to the next generation of testing. One such example is the analysis of stachyose content in soybean. Stachyose is the predominate member of a class of galactose containing oligosaccharides, classified as raffinosaccharides, found in relative abundance, approximately 5% by weight, in soybeans. Higher animals, including those monogastric animals raised as livestock or pets, lack the digestive enzyme necessary to cleave the bond among the monosaccharide units of these carbohydrates. As such, these sugars are not metabolically usable to the animal and, worse yet, they are believed to have a number of anti-nutritional characteristics. Through various plant breeding techniques and genetic engineering, it has been possible to essentially eliminate these raffinosaccharides from soybeans.

Stachyose is a particularly challenging analyte for NIR analysis because its chemical structure closely resembles both sucrose and cell wall carbohydrates, which are all abundant in soybeans. Despite having numerous samples ranging from 0 to over 5% stachyose, we have been unsuccessful in developing NIR transmittance based calibrations, and have an NIR reflectance calibration which we regard as semi-quantitative; having an *SEP* of 0.44% stachyose. Figure 1 shows a comparison of NIR estimated stachyose content to that measured by the reference method of HPLC. This calibration, developed on a NIRSystems 6500 instrument, was fit by modified PLS on the first derivative. Despite the obvious lack of analytical accuracy with the calibration, we still

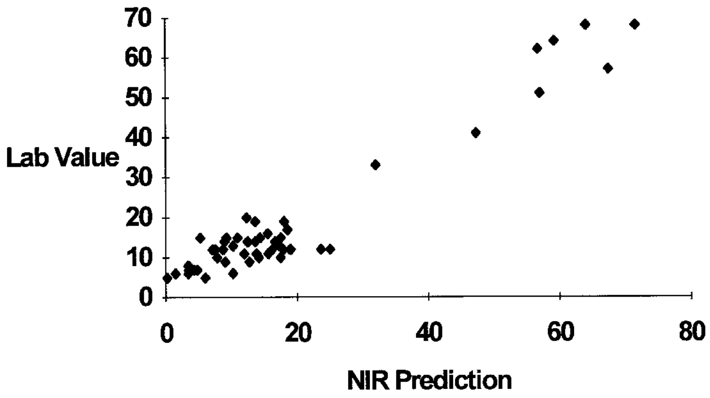

Figure 1. Prediction of Stachyose by NIR.

find it very useful as the primary objective is to rapidly screen those individuals with reduced stachyose content.

Oleic acid in soybean is an excellent example of the combined utility of NIR transmittance and NIR reflectance. Common soybean oil is very high in polyunsaturated fatty acids, typically 60% of the total fatty acids. While polyunsaturates have desirable functional properties, high levels of polyunsaturates impart high temperature oxidative lability to the oil. To improve performance in frying uses, a reduction in the levels of polyunsaturated fatty acids and a concomitant increase in the level of oleic acid, a monounsaturated fatty acid, is desirable. Through induced mutations and genetic engineering it has been possible to develop soybean varieties which produce oil having oleic acid in excess of 70% of the total fatty acids.

Because oil is a major component of soybean, approximately 15–20% of the weight, we were successful in developing a semi-quantitative calibration to oleic acid percentage by NIR transmittance. This calibration had an *SEP* of 3.6%. Far more accurate, however, was the calibration developed for NIR by modified PLS in the wavelength range of 1100–2500 nm. Figure 2 shows the predictive accuracy of both calibrations on the same set of test samples. In such a case we find it very useful to first analyze the samples as whole grain by NIR transmittance, and using broad selections made on this basis, proceed to a higher resolution of selection utilizing NIR reflectance.

One final example of the application of NIR analysis to the support of a biotechnology program relates to a program directed at the enhancement of essential amino acids in feedstuffs. Soybeans are the most commonly used source of essential amino acids in livestock diets. Most important among the essential amino acids is lysine. Normal soybeans contain approximately 2.5% lysine on a weight basis. To enhance the value of soybeans as a feed ingredient, we have utilized genetic engineering to double the content of lysine in soybeans.

Lysine is well suited for NIR analysis because its structure is unique among amino acids, having a terminal primary amine group. To investigate the potential for developing an NIR calibration to lysine content in these transgenic lines, a spectral comparison was made of soybeans with normal, moderately increased and very highly increased lysine contents. For this purpose, spectra from eight low, five intermediate and four very high lysine lines were accumulated. Spectra were averaged within each group. By averaging spectra for several samples of similar lysine content, spectral differences due to factors other than lysine content are reduced. The de-trended averaged spectra were overlayed to identify spectral differences consistent with the difference in

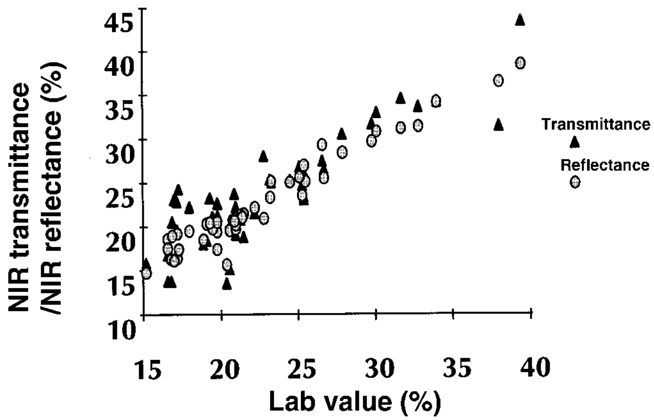

Figure 2. Comparison of NIR reflectance to NIR transmittance—prediction of oleic acid content.

lysine contents. At approximately 2100 nm, absorbance consistently increased with increasing lysine content (Figure 3). Pure lysine is known to have a strong absorbance in this spectral region.

Utilizing this spectral analysis as a guide, a calibration was developed by multiple regression on the first derivative. The best fit model utilized absorbances at 1684, 2100 and 2436 nm and had a *SEC* of 0.17% lysine for samples which ranged from 2.45 to 4.94% lysine.

Conclusions

As these examples should serve to demonstrate, near infrared spectroscopy is an ideal technique for the analytical support necessary to direct the development of crops with improved quality traits. Indeed, because of its rapid, low cost, high throughput, high precision nature; near infrared spectroscopy is a technique which is critical to the exploitation of biotechnology for improvement of agricultural products.

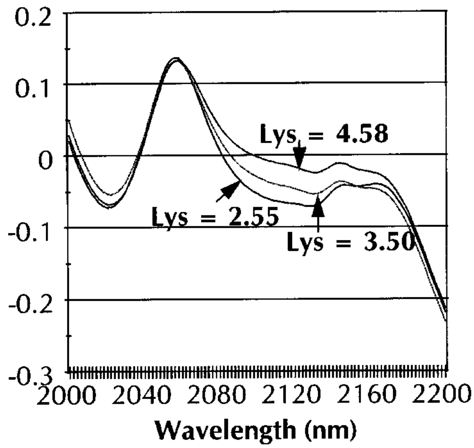

Figure 3. Spectral comparison of soybeans with differing lysine content.