Permanent samples for standardising near infrared instruments

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Introduction

While Grainspec Analyzers are manufactured to extremely high tolerances it is still necessary to "standardise" instruments so that they all produce as similar an optical response as possible. Recent studies on standardisation[1,2] have related largely to instruments which use sealed sample cups as reference samples.

With a whole grain analyser such as Grainspec, which fills automatically, standardisation is difficult as the difference between instruments relates partly to tolerances in the optical system and partly to differences in sample presentation. Freely flowing grain produces a chaotic sample packing system where the configuration of the grain differs from subsample to subsample and it is impossible to present the same physical sample to two instruments in the same configuration. Even given these limitations, Grainspecs predict moisture, protein and oil content of cereal and oilseeds with a high degree of accuracy.

Grainspec standardisation involves passing representative samples through every new "slave" instrument and, at the same time, through a "master" instrument which is used as a standard. Regression analysis is then used to correct the response of a slave to that of the master.

GrainBlocs[3] were devised as an alternative standardisation tool. They consist of grain held in a matrix of polyester resin making it possible to fix exactly the optical path so that, when measured on different instruments, the blocks become optical standards presenting the same light path in a way that is not possible with free grain. This eliminates the sampling error associated with whole grain allowing standardisation to be achieved with fewer samples.

Materials and methods

A GrainBloc is made by packing a mould, whose height and width at 50 mm fit exactly a Grainspec optical cell, with a sample of clean dry grain. A sheet of wire gauze is then placed over the top of the mould to prevent any grain escaping when resin (EM400PA shallow-cast resin, Trylon Ltd, Wollaston, Northants NN9 7QJ, UK) is added. A vacuum is then applied which encourages air bubbles to leave the grain/resin matrix. After five minutes the vacuum is removed allowing atmospheric pressure to force resin into any small air pockets still remaining in the matrix. The mould is then "topped up" with resin and the vacuum reapplied.

This process is repeated at least twice more before the wire screen is removed and the resin block is allowed to cure overnight before being cut into slices appropriate for the pathlength needed for the crop involved. Because the refractive index of resin is different from air it is necessary to

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adjust the pathlength of the GrainBloc to obtain the same response as free grain. A thin resin is then used to seal the exposed face of the block where it has been cut. A black anodised mounting plate, complete with a handle, is screwed and glued to the base of the block allowing it to be held firmly while the bottom shutter of the Grainspec is opened to insert the block into the sample chamber.

GrainBlocs are allowed to complete the curing process for a period of at least a week before being measured several times over a period of two weeks to check that the spectra are repeatable and stable. They are then made up into sets with differing spectral absorbance levels ready to be used as spectral standards for checking instruments in the field or for standardising slave instruments.

Results and discussion

Subsample spectra from a typical sample of barley and a GrainBloc are shown in Figure 1 (in both Figures 1 and 2, GrainBloc spectra are shifted to lower absorbance values for clarity). Because grain packs in a chaotic manner, subsample spectra are variable in terms of the amount of energy which passes through the grain to the detector system. This is equivalent to having a variable pathlength. Although there appears to be a single GrainBloc spectrum there are, in fact, three subsample spectra all superimposed upon each other. This is a direct consequence of the fact that the detector sees exactly the same sample of grain each time.

Figure 2 shows mean spectra of a sample of barley and a GrainBloc measured on the master instrument on three occasions. The three GrainBloc spectra show much smaller differences than those for free grain. If, when using the same instrument, we can be more confident of obtaining the same mean spectrum for a sample on subsequent occasions it makes the standardisation process more reliable.

The stability of GrainBlocs over time has important consequences in that it makes possible standardisation "at a distance". Difficulties associated with moving grain samples while maintaining them under the same conditions of temperature and humidity make it necessary to bring both master and slave to the same location. GrainBlocs can, however, be easily transported in a small sealed container from site to site. Instruments can then be standardised with minimal effort. GrainBlocs can also be used as "check samples" to monitor instrument performance over time.

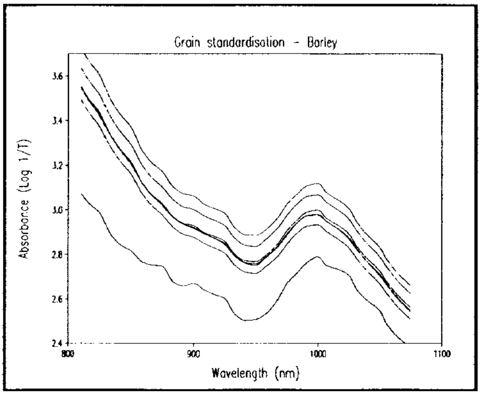

Figure 1. Subsample spectra from a sample of barley (upper dashed lines) and of a Grainbloc (lower solid lines).

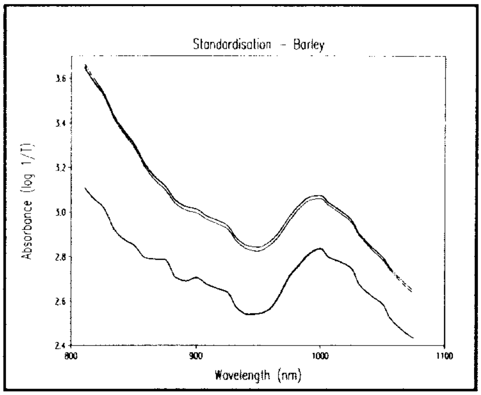

Figure 2. Mean spectra of a sample of barley (upper dashed lines) and a GrainBloc (lower solid lines) scanned on three occasions.

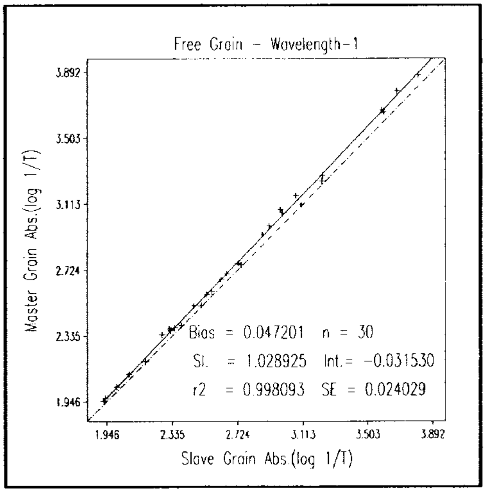

Figure 3. Standardisation plot for one of 33 wavelengths measured on a slave and master Grainspec using free grain.

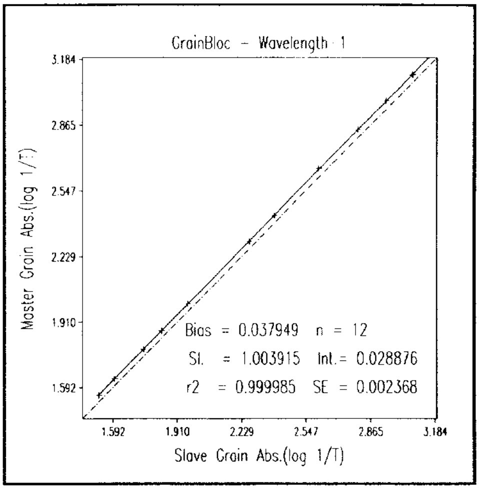

Figure 4. Standardisation plot for one of 33 wavelnegths measured on a slave and master Grainspec using GrainBlocs.

Standardisation requires, for every wavelength, the calculation of a slope and intercept term correcting the spectral response of a slave to that of the master instrument. Because manufacturing tolerances are small, slopes are always close to 1.00 and intercepts are always close to 0.00. It has been shown that any increase in error associated with standardisation is detrimental to the performance of Grainspec in the field and, as a consequence, any improvement in standardisation is desirable. Figures 3 and 4 show slopes and intercepts for regression lines relating the response of two instruments at one of 33 possible wavelengths using free grain and GrainBlocs.

With perfect optical systems in both instruments, all points should lie in a straight line. With free grain (Figure 3) the exact slope of the regression line needed to correct the response of the slave instrument is more difficult to estimate accurately as the points do not always lie on the line. To obtain the best possible estimate it is necessary to use a minimum of 30 samples.

Measurements obtained using GrainBlocs (Figure 4) are more reproducible and all lie close to the regression line. Both the bias and Standard Error associated with the regression are improved by an order of magnitude. As a consequence, we obtain a much better estimate of the regression line needed to standardise this wavelength with many fewer samples thus saving time in testing.

Conclusions

While GrainBlocs do not have exactly the same spectral response as free grain, they have a spectral shape which is similar enabling them to be used as standardisation samples which can be used remotely at any site. In addition, they can be used as standard samples to check the performance of instruments in the field.

References

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2. J.S. Shenk, M.O. Westerhaus and W.C. Templeton, *Crop Sci.* **25,** 159 (1985).
3. GB Patent Application No. 9504976.3.