

Validation of Near Infrared (NIR) Spectroscopy vs Milling Index Method

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Introduction and methods used

A near infrared (NIR) transmission calibration has been used to estimate the milling Index (MI) of whole maize kernels of an independent data set (n=330). The actual MI of these samples has been determined using the reference method, i.e. the Roff MI. The current data set included the 87 samples used to compare these two methods in 2007 for which a report has been submitted by myself. It is believed that the remainder of the data set comprised data obtained from samples from 2005-2009. The NIR and Roff MI results, as supplied by the ARC Summer Grain Centre, will be evaluated and reported on in this report.

Statistical and data analysis

Analysis of variance (ANOVA) has been performed to compare measurements as obtained by the Roff MI and NIR spectroscopy methods, respectively, to determine absolute differences. Additionally the intra-class correlation coefficients (ICC) have been determined as the ICC agreement that correlates measurements with each other while also taking into account the differences in absolute values of the respective measurements and the ICC consistency that correlates measurements with each other, but ignores absolute differences. The standard error of prediction (SEP) and bias has been calculated to evaluate the NIR spectroscopy calibration.

Results and Discussion

The descriptive statistics of the Roff MI reference data and the validation statistics of the estimated NIR MI vs the Roff MI results are listed in **Table 1**.

Table 1 Descriptive statistics of Roff Milling Index reference data and validation statistics of NIR Milling Index calibration

| Parameter | Roff Milling Index | NIR Milling Index |
|--|--------------------|-------------------|
| n | 330 | 330 |
| Mean | 93.66 | 118.53 |
| SD | 12.52 | 12.69 |
| Minimum | 50.48 | 72.70 |
| Maximum | 127.99 | 145.47 |
| Standard error of prediction (SEP) | | 25.689 |
| Bias | | -24.875 |
| Standard error of prediction corrected for bias (SEPC) | | 6.656 |
| r | | 0.870 |
| R ² | | 0.760 |
| ICC consistency | | 0.870 |
| ICC agreement | | 0.295 |

$$\begin{array}{ccc}
 \text{SEP} = & \text{SEPC} = & \text{Bias} = \\
 \sqrt{\frac{\sum_{i=1}^n \left(y_i - \hat{y}_i \right)^2}{n}} & \sqrt{\frac{\sum_{i=1}^n \left(y_i - \hat{y}_i - \text{BIAS} \right)^2}{n-1}} & \frac{1}{n} \sum_{i=1}^n \left(y_i - \hat{y}_i \right)
 \end{array}$$

The correlation plot of the Roff MI measurements vs the estimated NIR spectroscopy MI measurements are shown in **Figure 1A**. **Figure 1B** depicts the significant difference ($P = 0.00$) between the Roff MI and NIR spectroscopy MI measurements. From the ICC consistency (0.870) it is clear that there is a correlation between the obtained results, but the ICC agreement (0.295) indicates that there are differences in the absolute values of the two respective measurements. This is also evident from the clear bias observed in **Figure 1A**.

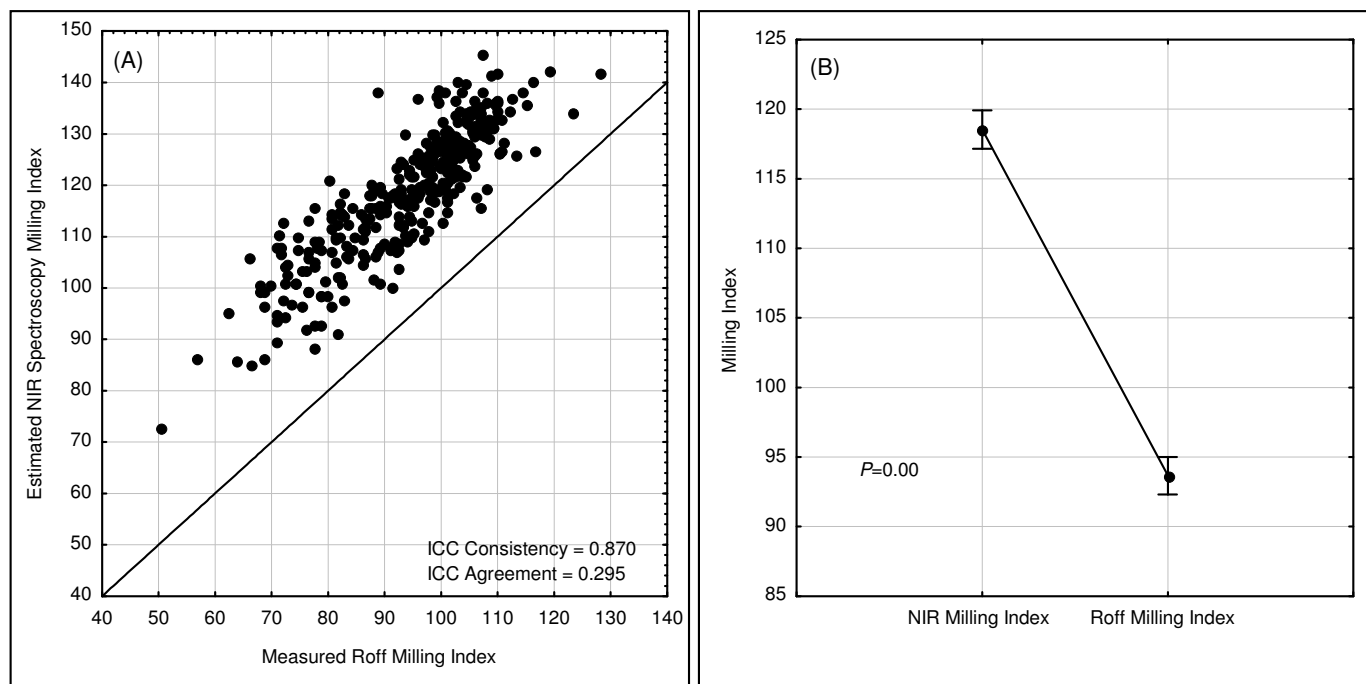


Figure 1 (A) Intra-class correlation (ICC) consistency plot of the Roff Milling Index values vs the NIR spectroscopy Milling Index values and (B) significant difference between the Roff MI and NIR spectroscopy MI measurements.

This standard error of prediction (SEP) for this data set was determined as a MI value of 25.689. The SEP can statistically be interpreted as the expected error, with a probability of 68%, to be within an interval of $\pm 1 \times \text{SEP}$ and with a probability of 95% within an interval of $\pm 2 \times \text{SEP}$. This means that 68% of all the results can be expected to have an error of 25.689 or 95% of all the results, an error of 59.378. If a bias (-24.875) correction could be successfully applied then these values will reduce to 6.656 and 13.312, respectively. The SEP should be as small as possible or as close as possible to the error of laboratory (SEL). The SEL can be calculated if duplicate reference measurements have been made. There is thus no rule as to how small the SEP should be and it is for the user to determine the acceptable error for a specific application.

The results obtained with the current data set agrees with the results obtained and reported in 2007. It thus seems that the same calibration (a large bias was also observed in 2007) is still being used. It is thus crucial that the source of this large bias being investigated and removed if possible. Alternative the calibration should be updated/recalculated with new data. It is this large bias that resulted in the small ICC agreement which confirms that there is a difference in the reference MI values and estimated NIR MI values.

Conclusion

Reasonable coefficient of determination (R^2) and ICC consistency values have been obtained during the validation of the current NIR spectroscopy MI method for predicting the MI in whole maize samples.

However, the large observed bias resulted in a low ICC agreement and the subsequent low accuracy (high SEP). As reported previously this is a great cause for concern as due to this large bias the current estimated NIR MI results should be used with caution. It is therefore advised, again, that the source of the bias is investigated and understood and a method being developed that would greatly reduce this error. It might be necessary to obtain a small sample set to be measured in advance (also by means of the reference method) to determine the bias correction before any future, unknown samples are measured. To improve the robustness of the calibration the samples used for this validation could be included in the previous calibration set and the calibration recalculated and revalidated. This should result in a prediction model suitable for estimation of MI of whole maize kernels using NIR transmission spectroscopy.