FINAL REPORT
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PROJECT TITLE
Evaluation of hectoliter mass equipment in order to recommend a suitable device for determination of hectoliter mass of maize in South Africa

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Objective

The objective of this project was to evaluate different hectoliter mass (HLM) measuring devices used in Australia, Canada, France, Germany, United Kingdom and North America compared to the chondrometer, currently used in South Africa for HLM determination of wheat, in order to recommend a suitable method and device for the determination of HLM of maize for the maize industry in South Africa.

This final report includes the results on the effect of removal of impurities (dockage) from maize samples on HLM measurements as well as the potential use of near infrared spectroscopy (NIRS) to predict HLM values of maize samples.

Introduction

From the results reported earlier (Final Progress Report, November 2006) it was concluded that the repeatability of the measurements within each HLM device was similar between the respective devices. It was then observed that the HLM measurements performed on the HLM devices from Australia, France and Canada resulted in significantly higher values than those obtained from the other devices. Measurements performed on devices from Australia and France resulted in HLM values significantly higher that those obtained from the device from Canada. No significant differences were observed in the HLM measurements obtained from the remaining devices. These results were obtained on maize samples that have not been cleaned before determination of HLM values. It has, however, been shown that although actual differences in HLM values were observed the HLM values obtained from the respective devices do correlate with each other making the use of correction factors a possibility for comparing results if obtained from difference devices. In terms of practicality it was only the device from France that was not suitable for HLM determinations of maize. The opening of the funnel seems to be too small to allow the maize kernels to flow freely into the measuring cylinder.

Materials and Methods

Comparison of the different HLM devices using samples before and after impurities have been removed

Maize samples were kindly supplied by Tiger Brands, Sasko Grain, Godrich Flour Mills, Noordfed, Ruto Mills and the SAGL. Ten samples were selected to cover as wide a range of HLM values as possible. The 10 maize samples (before and after impurities were removed) were used to evaluate hectoliter mass devices from different countries as listed in **Table 1** compared to the South African chondrometer of which two were evaluated.

Table 1 Hectoliter mass devices evaluated compared to the South African chondrometer.

Country	Equipment
North America	Filling Hopper with quart cup and strike-off stick
Canada	Cox Funnel with 0.5 liter measure and wooden striker
Australia	Aluminium 0.5 liter with filler and cutter bar
Germany	KERN 220/222 Grain samples
United Kingdom	Farm-Tec Easi-Way Hectoliter Test Weight Kit
France	Nilema Liter

The maize samples were poured through a Boerner Seed Divider twice in order to obtain a well-mixed sample. Each sample was divided into three, 1.5 kilogram sub-samples. The HLM determination of each sample was always determined on the USA device first, as this device requires the largest sample. The testing order of the other devices was randomly chosen. The work sample obtained from the American device was subsequently used to measure the HLM on the other devices, as well. Duplicate tests were executed on each HLM device with the same work sample.

The impurities of each of the ten 1.5 kg maize samples were subsequently removed by means of a standard maize grading sieve (6.35 mm round-hole sieve). After the respective cleaned maize samples were thoroughly mixed the HLM determinations were performed again in duplicate as described earlier.

Operating procedures for the different hectoliter mass devices

The HLM determinations were performed using the respective devices as described in the Final Progress Report (November 2006).

Statistical analysis

Analysis of variance (ANOVA) of repeated measurements has been performed to determine statistical difference between samples before and after removal of dockage.

NIRS spectral acquisition and calibration development for prediction of HLM values

A Büchi NIRLab N-200 Fourier transform near infrared (FT-NIR) spectrophotometer with NIRLabWare (version 3.0) near infrared (NIR) measurement software was used to perform the NIRS measurements in diffuse reflectance mode on 145 maize samples. The HLM values were determined using the HLM device from the UK in duplicate on 1 kg maize samples. The whole grain maize samples were subsequently presented to the instrument in rotating glass petri-dishes and the NIR spectra collected from 1000-2500 nm at a resolution of 8 cm^{-1} resulting in 1557 data points.

The Unscrambler (version 9.2) was used for calibration model development. The spectral data were pretreated with multiplicative scatter correction, followed by Savitsky-Golay first derivative pretreatment (segment of 15). The partial least square (PLS) regression calibration model was validated by means of full cross validation.

The accuracy of the calibration model was expressed by means of the standard error of calibration (SEC), standard error of cross validation (SECV), the coefficient of determination (R^2) and the ratio of SECV to standard deviation of the validation set (RPD), which is an indication of the efficiency of a calibration according to the equations as listed in **Table 1**. The goal of model development is to obtain a calibration model with a low SECV, a high R^2, preferably above 0.96 and a RPD higher than 3. The SECV should also be as close as possible to the standard error of laboratory (SEL).

Table 1 Equations used to calculate NIRS calibration statistics.

Statistic	Equation	Recommendations
SD[a]	$\sqrt{\sum y^2 - \frac{\left[\frac{\left(\sum y\right)^2}{n}\right]}{n-1}}$	
SEL[b]	$\sqrt{\frac{\sum\left(y_1 - y_2\right)^2}{2n}}$	As small as possible
SECV[c]	$\sqrt{\frac{\sum_{i=1}^{n}\left(y_i - \hat{y}_i - BIAS\right)^2}{n-1}}$	As small as possible or close as possible to SEL value
BIAS[d]	$\frac{1}{n}\sum_{i=1}^{n}\left(y_i - \hat{y}_i\right)$	As close to zero as possible
R^2[e]	$\frac{\sum_{i=1}^{n_s}\left(\hat{y}_i - y_i\right)^2}{\sqrt{\sum_{i=1}^{n_s}\left(y_i - \hat{y}\right)^2}}$	Ideally 0.96 and higher
RPD[f]	$\frac{SD_{\hat{y}}}{SECV}$	Ideally 3 and higher

[a] Standard deviation, [b] Standard error of laboratory, [c] Standard error of cross validation, [d] Bias of the validation set, [e] Coefficient of determination, [f] Ratio of standard error of performance to standard deviation, y = reference value, $\hat{y}$ = predicted value, y_i = reference value for the i^{th} sample, $\hat{y}_i$ = NIR predicted values for the i^{th} sample, y_1 and y_2 = duplicate reference values, n = number of samples, t = number of terms in the model

Results and discussion

Comparison of the different HLM devices using samples before and after impurities have been removed

Figure 1 shows the least square mean plot after ANOVA of repeated measures have been performed. From the ANOVA it is clear that the HLM devices from Australia, Canada and France showed significant different results ($p<0.01$) compared to the other devices as has been found in earlier experiments. There was a statistically significant difference ($p=0.03$) between the HLM results of the respective samples before and after removal of dockage (**Figure 2**) with the difference between the mean values being 1.37 kg.hl^{-1}. No significant interaction ($p=0.31$) between the devices and the effect of the removal of dockage was observed (**Figure 3**).

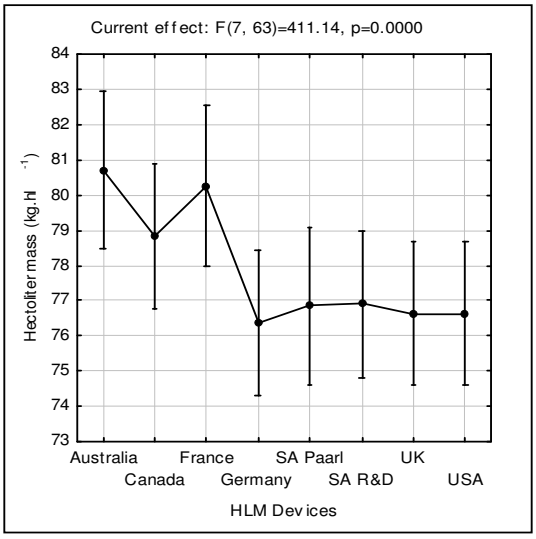

Figure 1 Least square mean plot after ANOVA of repeated measures have been performed showing differences between different devices.

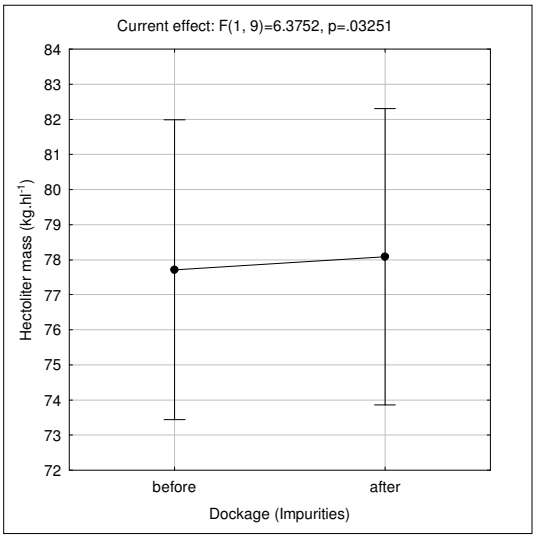

Figure 2 Least square mean plot indicating differences between the HLM measurements before and after removal of dockage

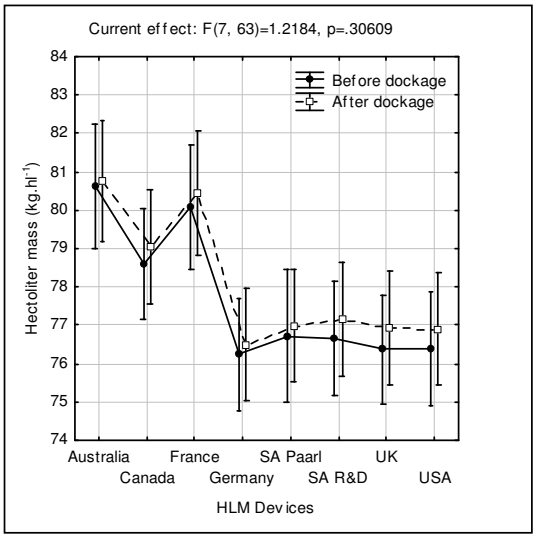

Figure 3 Least square mean plot indicating no interaction between the HLM measurements obtained form the different hectoliter mass devices on maize samples before and after removal of dockage.

The significantly higher HLM values obtained using the devices from Australia, Canada and France have been confirmed again during this experiment. The statistically significantly difference observed between HLM values before and after removal of dockage needs to be interpreted keeping in mind the levels of HLM on which remuneration and/or quality determinations might be based on in future.

NIRS spectral acquisition and calibration development for prediction of HLM determinations

Prediction HLM values from NIRS spectra was not as successful, but did show some potential (SEC = 0.76 kg.hl^{-1}; SEP = 1.13 kg.hl^{-1}; R^2 = 0.65; Bias = -0.0168 and RPD = 1.67) as shown in **Figure 4**.

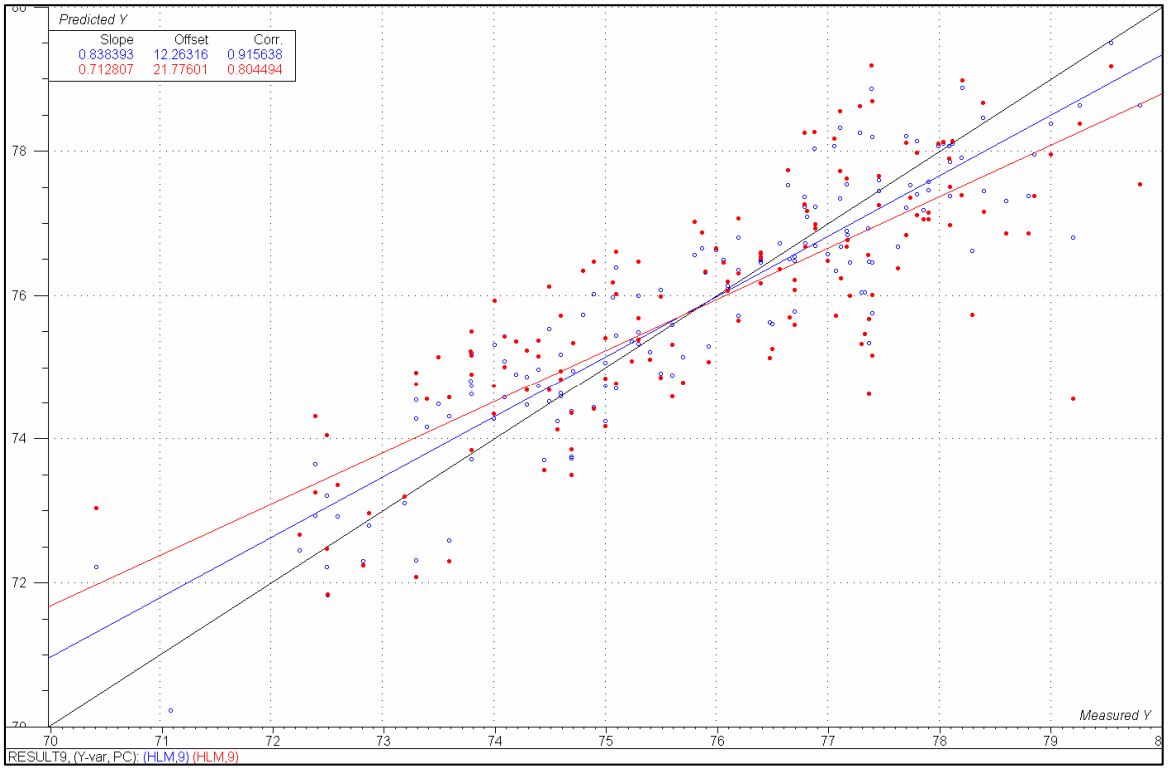

Figure 4 Calibration (blue/open) and validation (red/solid) scatter plot of NIRS PLS calibration for predicting HLM determination of whole maize kernels using 9 PLS factors.

The residual (difference between the reference and NIRS HLM results) has been used to calculate the standard error of prediction (SECV) according to the equations as listed in **Table 1**. The SECV can statistically be interpreted as the expected error, with a probability of 68%, to be within an interval of ±1SECV and with a probability of 95% within an interval of ±2SECV. This means that 68% of all the results can be expected to have an error of 1.13 or 95% of all the results an error of 2.26. This value should be as small as possible or as close as possible to the error of laboratory (SEL) which has been 0.26 kg.hl^{-1} in this case. There is thus no rule as to how small the SECV should be and it is for the user to determine the acceptable error for a specific application. Guidelines for interpretation of the R^2 in terms of NIR calibrations are given in the **Table 2**. The R^2 obtained for this current calibration (0.64) is therefore only suitable for rough screening of samples.

The obtained results can also be expressed in terms of the RPD, which is the ratio between the SEP to the standard deviation (SD) of the validation set. According to the interpretation guidelines in **Table 3** the RPD of 1.67 do not suggest this calibration to be suitable.

Table 2 Guidelines for interpretation of R^2 (adapted from Williams, 2001).

R^2 value	Interpretation
Up to 0.25	Cannot use in NIRS calibration
0.26-0.49	Poor correlation. Investigation is necessarily
0.50-0.64	Can be used for rough screening. More than 50% of variance in *y* (NIR data) accounted for by *x* (reference data)
0.65-0.81	Can be used for screening and some approximate calibrations
0.82-0.90	Can be used in most applications but with caution. More research is necessary
0.91-0.96	Can be used in most applications, including quality assurance
0.97+	Can be used in any applications

Table 3 Guidelines for interpretation of RPD (adapted from Williams, 2001).

RPD value	Classification	Application
0.0-2.3	Very poor	Not recommended
2.4-3.0	Poor	Very rough screening
3.1-4.9	Fair	Screening
5.0-6.4	Good	Quality control
6.5-6.8	Very good	Process control
8.1+	Excellent	Any application

Budget and expenditure Statement

Please find attached the final financial statement.

Conclusions

HLM values of maize samples measured before and after the impurities have been removed from the samples showed a low level of significance indicating that the current procedure of not cleaning maize samples before HLM is determined can be continued with. From this and previous results higher HLM values were obtained from the devices from France, Australia and Canada. However, as the results of the respective results do correlate with each other correction factors can be applied if comparisons are necessary between results obtained from different devices. It was only the device from France that was not suitable to measure the HLM of the maize efficiently due to the large size of the maize kernels. The South African chondrometer can therefore be used to measure HLM of maize, keeping in mind the differences of the three devices from France, Australia and Canada, respectively.

NIRS calibrations to predict HLM has been attempted for interest sake, but the current calibration obtained would only be suitable for rough screening.

Acknowledgements

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