Report on maize hardness testing method project

(Final feedback towards funding to support hardness project executed by Dr Glen Fox)

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Summary

Maize is an important part of the South African economy with white maize used primarily in the human food market. Currently, there are no quality standards used to trade maize, except for physical standards of screenings and hectolitre mass. Kernel hardness is an important characteristic that impacts on processing, with softer kernel producing a higher level of 'thrus' thereby reducing the efficiency of the milling process.

This study investigated the development of a hardness testing method. To gain an in-sight into maize hardness, a review was carried out on previous testing methods as well as looking into the physical and biochemical maize components that impact on hardness. A specific method, i.e. particle size index (PSI) was selected and used to assess a set of commercial hybrids that also underwent commercial milling. There was a moderate correlation between thrus and hardness using the PSI method, the Roff Milling Index (RMI) method as well as protein content. In addition, the method was used as a reference method to develop a near infrared (NIR) calibration for the rapid and non-destructive estimation of maize hardness. A set of samples from the National Cultivar Trials was used to develop the calibration. The calibration results support previous studies showing NIR is a reliable technology for estimating hardness.

Hardness is related to protein and results from the NIR study also support the underlying positive impact protein has on kernel hardness. Specific NIR spectral regions are highly influenced by protein and some wavelengths (1910 and 2140 nm) were also related to hardness.

Genetics and growing environment impact on quality. A statistical analysis of a limited data set, with the RMI method used as the testing method for hardness, suggested both genetic and environmental influences. However, there was a large error component. Due to the structure of the trials, the limited number of cultivars grown over multiple seasons and hardness testing, it was not possible to accurately partition the genetic, environmental or even method effects on hardness. Further studies based on appropriate statistical design with accompanying protein data would provide a solid basis for directing the breeding efforts and possible classification of South African maize quality.

Background

Maize is the most important summer crop grown in South Africa. Of the approximately eight million tonnes produced annually, its primary use is for human food although it is also used for animal feed. A major portion of white maize grown is used to produce maize meal. Production of maize meal through commercial mills produces a large amount of waste product. The commercial industry sees this waste as costly and inefficient. The quality of the maize used for maize meal production is selected primary on size and weight. However, the hardness of the endosperm itself could impact on the processing. This effect has been seen in flours produced from others cereals. Both wheat and durum required a hard endosperm texture if being used for bread, noodle or pasta flours.

In a recent review, Fox and Manley (*1*) described previous methods for testing maize hardness as well as the chemical influences. Hardness is an easily measured characteristic. There are a number of destructive methods that are based mostly on the resistance of the endosperm to grinding or abrading. Other methods include a measure of the particle sizes produced from a grinding method, the percentage of kernels that float in a solution with a specific density or a measure of the starch gelatinisation properties.

The methods used to assess hardness provide an indication of endosperm texture and possible end-use performance. However, the methods do not provide any insight to the physical or chemical components within the kernel that would be influencing kernel hardness. Starch is the main chemical component in maize (>70% db), while protein content can account for approximately 10% (db). However, both the content and composition play a major role in influencing hardness. The protein content itself is influenced by the environment in which the maize is grown. It is also controlled by the genetics of that specific maize cultivar. However, very few studies have reported on a detailed genetic and or environmental study for maize hardness. In South Africa, early work by Hohls (*2*) clearly showed both genetic and environmental effects on a diallel maize cross for maize hardness. However, unlike the winter cereals, maize hardness is not controlled by a single gene family. Sene (*3*) has shown that the genetic regions across a number of chromosomes for maize endosperm texture are linked to regions for kernel weight, protein and starch.

As mentioned earlier, while the methods to measure hardness indicate the physical hardness, they do not necessarily provide information on the controlling mechanisms. Near infrared (NIR) spectroscopy is a non-destructive method that estimates hardness (and other traits) based on a mathematical model using data from a reference method and NIR wavelengths. Hence, a calibration can be developed to simply estimate hardness. However, the NIR spectra can also provide information on the chemical components that may be influencing hardness and the resultant calibration.

Of particular interest for our project, was a method developed specifically in South Africa (ARC Summer Grain Centre, Potchefstroom), namely the Roff Milling Index (RMI). This method was developed to simulate commercial milling processing. In addition, an NIR calibration was developed on an Infratec (FOSS) NIR transmission instrument (ARC Summer Grain Centre, Potchefstroom). These calibrations were made available to a number of laboratories. However, the maintenance of the calibration is critical to the successfully application of the calibration by the breeders to select for maize hardness or application by industry to grade maize for hardness.

The objective of this study was to develop an alternative, but still suitable reference method for maize hardness using samples from either commercial or breeders' trials to develop an NIR calibration that could be easily maintained.

Methods

Hardness method

After an extensive review of the literature, and based on available resources, a particle size index (PSI) method was considered as the most suitable. This was due to the simplicity of the method as well as the use of smaller sample sizes (50 g of whole kernels). The PSI method uses a grinding and sieving process to determine the particle size distribution. A Cyclone mill with a 1 mm sieve was used to grind 50 g of maize kernels. The resultant flour (10 g) was then sieved using a Retsch Rotap Sieve Shaker (**Fig. 1**) through two sieves. The sieve sizes used were 150 µm and 75 µm,

with a pan to catch particles less than 75 µm. Samples were sieved for 10 min. The shaker could hold two sets of sieves and pans, therefore duplicate tests were performed simultaneously.

Figure 1 The Retsch Rotap Sieve Shaker with duplicate sets of sieves.

Samples

Initially, a small set (eight) commercial hybrids were tested for hardness using the PSI method. These samples were grown at two field sites. The harvested grain was then bulked for commercial milling assessment. Prior to this we obtained individual samples from the field plots. As well as the data from the PSI method, data from a number of other hardness or hardness related methods were also obtained. These methods were thousand kernel weight (TKW), percentage of floaters in a sucrose solution, and NIR absorbance at 1680 nm. Grain protein (GP) and Roff Milling Index (RMI) data was also made available (Anita Ybema, ARC Summer Grain Centre, Potchefstroom).

In addition, a statistically study was carried out using RMI results from two previous years' trials (2003/04 and 2004/05 National Cultivar Trials) to ascertain any genetic and or environmental effects on hardness.

NIR methods

Two NIR instruments were used to assess the potential to develop NIR calibrations. These instruments were a Büchi NIRFlex N-500 and Bruker MPA (**Fig. 2**). These were FT-NIR instruments with a spectral range of 1000 to 2500 nm (4000 to 12000 cm^{-1}). These instruments scan in approximate 2 nm increments. Both whole grain and flour samples were scanned. Spectral data from the Büchi NIRFlex N-500 was exported to The Unscrambler (v9.1) while for the Bruker MPA the Bruker software Opus (v6.5) was used. Each calibration set consisted of 120 samples and the calibration models were validated using full cross-validation. The Instruments were kindly made available to us by Büchi Labortechnik AG, Flawil, Switzerland and Bruker SA, Johannesburg.

Figure 2 Images of the Büchi NIRFlex N-500, the Bruker MPA and the author (dr Glen Fox).

Results

Commercial milling trial

The first set of samples tested using the PSI method were eight commercial hybrids grown for a commercial trial. These eight hybrids were grown at two localities, but were bulked for the commercial trials. In terms of statistical analysis and field variation that could have impacted on the

results it would have been better if the two field plots were not bulked. The results from the quality analyses of the individual samples suggested there were some differences in quality due to locality. Data supplied by ARC Summer Grain Centre shows considerable variation in protein content between the samples for each hybrid. Hence bulking grain differing in protein content could have influenced hardness and therefore processing in the mill. Data from the commercial milling process was provided by Colin Wootton. According to Colin Wootton better correlation with hardness was found with the commercial mill's degerminator thrus percentage rather than with the total offal percentage. Both sets of results were, however, supplied.

Table 1 shows the results from the commercial milling trial (thrus and offal with PSI, TKW, floaters, NIR absorbance at 1680 nm, GP and RMI). There was a range within all traits measured for the commercial hybrids. The results for hardness milling methods, PSI and RMI, as well as for GP, TGW and floaters, suggests variation between each of the two samples (different localities) for samples A, B, C, D and E.

While it was not possible to carry out an accurate correlation analysis of the individual samples from commercial milling trial with the data for all the other traits, trends were observed. As suggested by Colin Wootton, the percentage thrus was better correlated than percentage offal; percentage thrus were positively correlated to the RMI and density (**Table 2**). Since the RMI was developed to simulate commercial milling, this would be an expected relationship. The positive trend to floaters suggested the denser the kernels, the lower the level of thrus. This could be explained as denser endosperm, i.e. harder or more vitreous kernels would result in reduced percentage thrus in the mill.

Conversely, thrus were negatively related to protein and PSI. However, as there was a range in protein and hardness for each sample, bulking the hybrids has masked the contribution from individual samples in providing more accurate relationships. This is confirmed when PSI, GP and TKW were higher correlated. Harder kernels, generally have higher protein and were denser, and produced fewer small particles. These results suggeste protein content and kernel density have a strong influence on hardness and hence could influence milling efficiency.

Table 1 Results from commercial mill trial and laboratory analysis for eight commercial hybrids

Sample	Thrus (%)	Offal (%)	PSI	GP * (%)	TKW (g)	Floaters (%)	Abs 1680 nm	RMI*
A1/A2	26.7	38.4	41/39	10.9/8.0	41.7/36.7	56/44	0.3034/0.3093	96.9/82.1
B1/B2	17.6	25.7	47/45	11.8/9.7	47.5/37.9	15/8	0.3379/0.3331	110.0/106.1
C1/C2	20.4	36.5	37/37	11.4/8.3	47.4/35.3	26/20	0.3237/0.3164	100.6/88.6
D1/D2	26.4	39.9	38/39	10.5/8.4	49.4/32.6	40/23	0.3280/0.3221	101.5/94.5
E1/E2	21.2	32.6	37/37	9.7/7.8	38.4/36.4	23/22		99.8/91.2
F	23.5	41.8	42.6	8.5	34.3	20		102.3
G	Withdrawn		30.7	8.9	42.1	32		93.1

*Data supplied by ARC

Table 2 Correlation coefficients for hardness and protein content for eight commercial hybrids

Method	Thrus	Offal	PSI	GP	TKW	Floaters	NIRAbs	RMI
Thrus	1.0000							
Offal	0.8354	1.0000						
PSI	-0.5504	-0.5436	1.0000					
GP	-0.3872	-0.3379	0.7734	1.0000				
TKW	-0.1434	-0.2086	0.4826	0.8365	1.0000			
Floaters	0.8055	0.5825	-0.4600	-0.0276	0.2256	1.0000		
NIRAbs	-0.4260	-0.3998	0.2296	0.4179	0.5769	0.0601	1.0000	
RMI	0.5036	0.4770	0.9742	0.7192	0.4820	0.0436	0.2222	1.0000

Understanding hardness through genotype and environment

As part of gaining an understanding of maize hardness *per se* and how best to test maize hardness, we undertook a retrospective genotype x environment (GxE) statistical analysis. With assistance from Mr Thinus Prinsloo from ARC Summer Grain Centre, we obtained RMI results for the maize trials grown in two seasons (2003/2004 and 2004/2005). These trials were replicated and grown over multiple locations. However, due to the limited number of cultivars grown over both

seasons (only six), it was not possible to carry out an analysis over years. Hence, using results from each season, the results suggested genetic and environmental effects. It was, however, not possible to show any significant differences between sites or cultivars. In addition, we were unable to obtain yield or protein content data for these trials which limited the statistical analysis. If either of these traits were available we could have identified the level of within field variance which would have allowed a more accurate analysis. Further, the availability of protein content data would have provided one of the most comprehensive data sets for a correlation with RMI. This data would have provided a great platform to position the industry on understanding cultivar effects in relation to yield, protein and hardness as well as allow a calculation of heritability. The measure of heritability gives breeders an indication of the level of how heritable a trait.

It is suggested that the industry and breeders provide yield, protein content and hardness data (along with other kernel traits such as hectolitre mass) over multiple years and work with an experienced statistician to carry out an appropriate analysis. This will ensure the industry and breeders have a sound understanding of the genetic and environments effects on hardness and protein content. The relationship between protein content and hardness has been explored and described earlier. However, by using a large number of cultivars, grown over many localities and years, the data could provide information on the relationship between these traits on current and new South African maize hybrids. In addition, it could provide breeders with information on improving their breeding programs, either through understanding the phenotypes or using the phenotypic data for genetic analysis via molecular markers.

NIR calibrations using PSI as reference method

Developing NIR calibrations requires a number of steps. The calibration set must reflect the type of samples and range of values that will be estimated in future. The reference method should be a method with a low level of error and high level of precision (e.g. not influenced by operator). For traits such as protein or moisture contents, the reference methods are well documented and International Standard methods available. For wheat or durum, hardness methods exist using a PSI method or the Single Kernel Characterisation System (SKCS). Hardness methods for other cereals have not been standardised so methods using similar technology have been used to allow comparison of values or the use of instrumentation for multiple grain types. The method used in this study was based on previous methods used for maize and the well established method for wheat.

The NIR technology used were two scanning instruments (1000 to 2500 nm) with commercial software. The calibrations models developed suggest a high level correlation between the spectral and the reference data. For whole kernel maize the coefficient of determination (R^2) for the Büchi and Bruker instruments were 0.85 and 0.88, respectively. **Fig. 3** shows the regression plot for the actual and predicted values for whole grain scanned with the Büchi instrument. The level of error was low (standard error of prediction (SEP)) at 3.5 and 3.6, respectively, for the two instruments. The **R**atio of Standard Error of **P**rediction and Standard **D**eviation (RPD) was 2.3 and 2.6, respectively. Values above 2.5 suggest the calibration model could be used for screening in a breeding program. For maize flour, the R^2 values were 0.92 and 0.95 respectively, with RPD values of 3.1 and 4.0 for the Büchi and Bruker instruments, respectively.

The results indicated both the whole kernel and flour calibration models would be useful for screening breeding lines. The models would be improved with additional samples with a broader range of hardness. In addition, having the protein content data could help select samples based on the protein hardness relationship. Selecting samples based only on hardness could be limiting the spectral variation. NIR spectra are based on chemical bonds, i.e. C-H or N-H. The relationship between protein and hardness would be influenced by the N-H bonds in the protein as well as specific amino acids that control the protein structure and it's binding to starch. **Fig. 4** shows loading line plots with a major peak at 1900 to 1950 nm for both PCs 1 and 2. This region is associated with both protein and starch. A peak at around 2100 in PC 3 is also associated with protein. When using NIR spectra that is limited to the spectral region, 1900 to 2150 nm, to develop a calibration the R^2 for whole grain was only slightly lower (R^2 = 0.78) compared to using the full spectral range (1000 to 2500 nm).

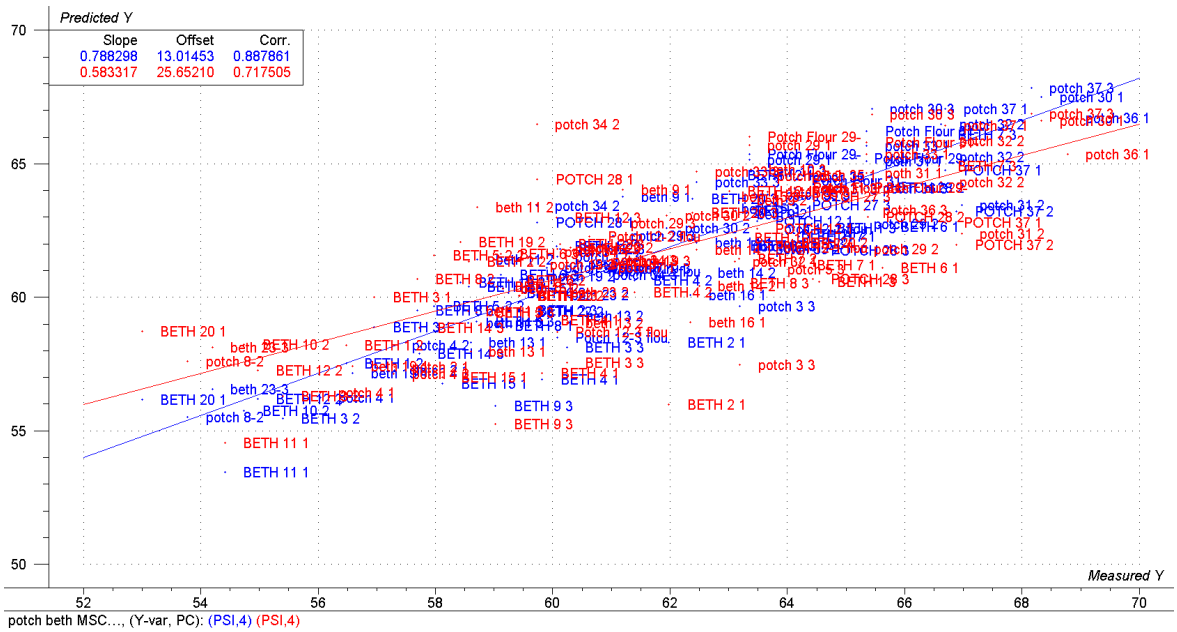

Figure 3 Regression plot for the actual and predicted values for whole grain scanned with the Büchi instrument.

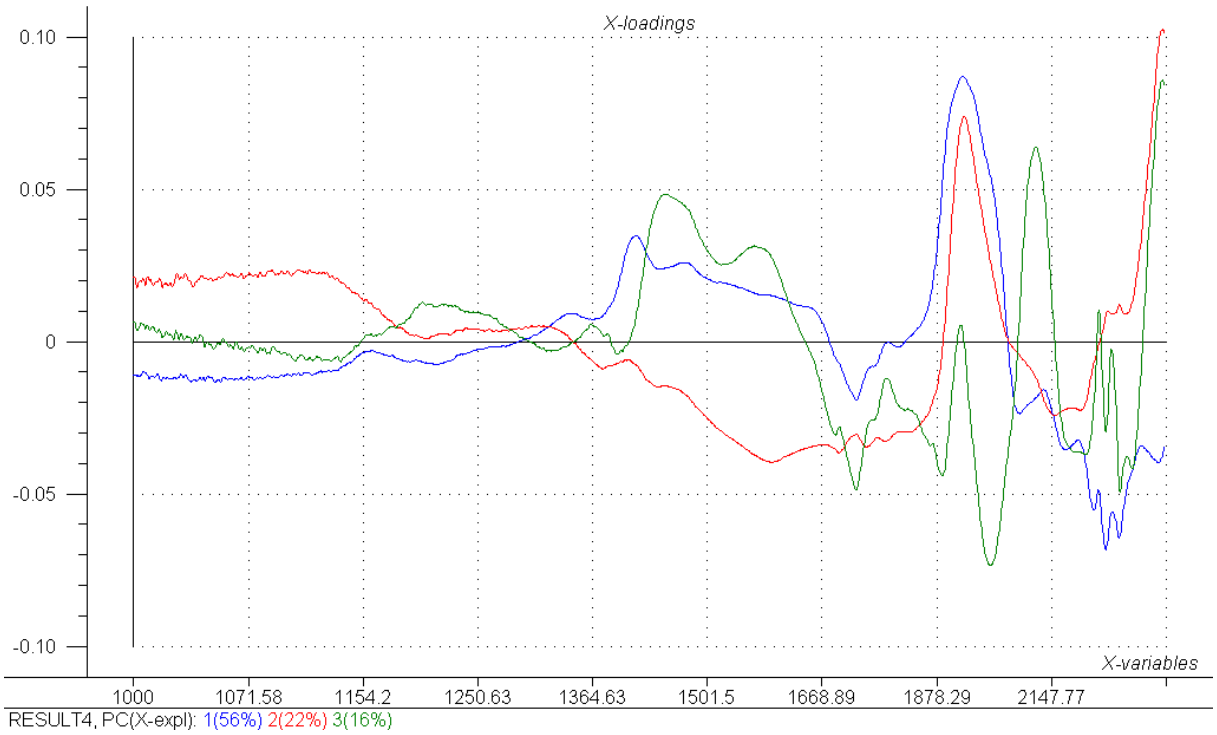

Figure 4 NIR loading line plots of the first three PCs for maize flour.

Understanding NIR spectral data in relation to hardness and protein

To further explore the hardness and protein relationship, we scanned (Bruker MPA) a specific protein fraction (zein) that had been isolated from maize (Kim O'Kennedy, MSc in Food Science student). The NIR spectrum for the zein sample (**Fig. 5**) shows that there are major peaks at around 1850 (with a trough at 1940) and 2100 nm. The peak around 1850 nm with the associated trough indicates starch-moisture interaction and the peak around 2100 nm is associated with N-H bonds (protein).

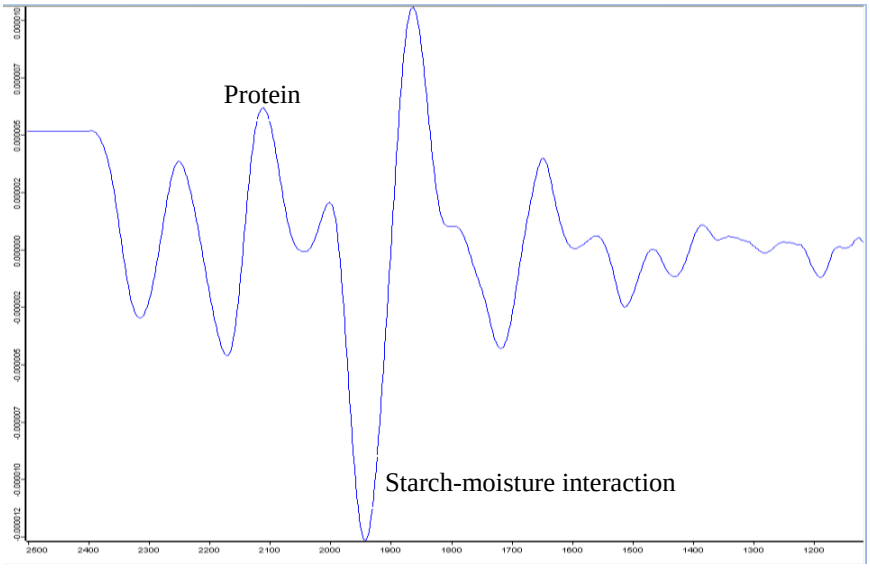

Figure 5 NIR spectrum of extracted maize protein fraction (zein).

Conclusion

This study showed the potential of the PSI method as a simple method of testing grain hardness using small sample sizes. The importance of well constructed experimental designs before studies commence is illustrated and stressed.

Outputs

1. Fox G & Manley M (2009) Hardness methods for testing maize kernels: a review. *Journal of Agricultural and Food Chemistry*, 57, 5647-5657 (DOI: 10.1021/jf900623w). (Impact Factor:2.532)
2. Fox GP, Manley M, Kidd M & Prinsloo T (2009) Genotype and environmental effects on hardness in maize (*Zea mays*) grown in South Africa. Combined Congress: South African Society of Crop Production; Soil Science Society of South Africa; Southern African Society for Horticultural Sciences; Southern African Weed Science Society, Stellenbosch, South Africa, 19-22 January 2009 (lecture).
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