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Improving mill efficiency through rapid analysis methodologies

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ABSTRACT

A rapid analysis system for measuring key constituents in sugarcane factory products has been developed using diode array (DA) near infrared (NIR) spectroscopic technology. Analysis of key constituents in prepared cane, bagasse, juice and syrup streams, magma, massecuite, molasses, raw sugar and mill mud is feasible. The benchtop NIR spectroscopic systems are mill laboratory-based and designed to augment the analysis already conducted by mill laboratories. More frequent analyses of mill factory products will allow Mill Engineers to minimise losses, and consequently, improve the factory's coefficient of work. Additional benefits accrue through improved final product quality and the ability to schedule maintenance based on impurity loading.

The NIR spectroscopic analysis technique has been rigorously evaluated to demonstrate acceptable performance under different conditions. Validation of systems in green-field sites has shown that the global models provide suitable turn-key functionality. Standard error of prediction (SEP) values show that mature and semi-mature models are accurate against the traditional wet chemistry models and repeatability and reproducibility statistics for the NIR spectroscopic technique are better than those published for equivalent wet chemistry methods in the Australian and International method manuals.

Characterisation of selected sugarcane factory products highlighted the chemical and spectral variability in the samples, but molecularly-targeted calibration models did not improve the predictive performance of the NIR spectroscopic technique. Despite this, NIR spectroscopic models for the analysis of fresh raw sugar were developed and its demonstrated performance has been equivalent or better than the standard model for evaluating pol and moisture in fresh raw sugar.

EXECUTIVE SUMMARY

Australian sugarcane factories rely on laboratory analyses of the intermediate and final products to monitor product quality and loss and define the overall efficiency balance of the factory. Over the last 20 years, the number of staff in mill laboratories has declined due to financial pressures in the modern production environment. This has resulted in few samples being analysed, which means Mill Engineers have less information available to decide control settings and maintenance procedures. Currently, for critical parameters, such as sugar pol and moisture, wet chemistry analyses may be conducted every shift, resulting in a feedback loop of three to ten hours. For less critical products such a C molasses, analyses may be conducted on a daily, or even weekly basis on composite samples. By the time information is available, thousands of tonnes of sugar have been produced.

This project aimed to improve the frequency of mill laboratory feedback by developing rapid analysis systems that are easy to use and maintain. The systems are capable of analysing multiple key constituents (e.g. brix, pol) in nine factory products. Each analysis takes less than one minute. The project was developed with strong industry linkages, which were maintained throughout the duration of the project with extensive mill trials to evaluate the technology. This was designed to allow the end users to experience the technology in their environment and expose them to the potential benefits. Additionally, this provided frequent feedback and performance monitoring for the project investigators and ensured the outputs and outcomes were end user-focussed.

Several outputs were produced in this project under the main umbrella of the product: the NIR spectroscopic analysis system, including soft technology, products, communication/capacity builders and tools/enablers. These outputs will speak to the following outcomes, identified in SRA's 2017-2021 Strategic Plan: a) Increased/improved mill laboratory output (Profitability), (b) Improved efficiency in the mill (Sustainability), (c) More sugar (Profitability), (d) Improved sugar quality (Sustainability and Profitability), (e) Improved safety (Sustainability), (f) Support for transition to true purity (Capability, Profitability and Sustainability), and (g) Strong collaborative networks (Organisational excellence).

Four of the systems developed during this project are already in use by three Australian sugar industry companies, indicating industry suitability and value. Assessment of the full economic benefit realised by this technology is difficult to quantify, however one user indicated that the system recovered its costs and more in a single use event.

Performance and acceptance standards for the traditional methods are not available within the Australian sugar industry, and hence a simple pass/fail assessment of the NIR spectroscopic methods is not feasible. Additionally, the technology can be used for many different applications, specific to a mill, with each having different analysis quality requirements. The user must evaluate whether the technique will be fit-for-purpose for their proposed application by evaluating the error thresholds and tolerances of the new and old methods against the potential for increased sampling frequency. Based on the validation data reported here, we are seeing adequate accuracy and precision to make informed decisions around factory operations, particularly for raw sugar, pan products and prepared cane.

The development of the calibration suite during this project has allowed Tully Mill to integrate the system into their routine operations. Additionally, Queensland Sugar Limited (QSL) have purchased and implemented two systems for routine use. During the 2017 season trial, Victoria Mill used the SRA NIR spectroscopic analysis system for routine monitoring of their sugar quality for export as well as pan stage monitoring and troubleshooting individual fugal performance. Several other mills are supportive of purchasing and using a system, but the timing is poor due to the extensive, industry-wide CAPEX replacement required for the Cane Analysis Systems (CAS).

Promotion and extension activities must continue beyond the project completion in order to capitalise on the momentum generated during this project. This will be conducted through the

commercial SRA NIR spectroscopy team, based in Meringa under the Milling Efficiency and Technology banner.

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1. BACKGROUND

Section 1 of this report will provide background information and context to the research project by explaining the project rationale and its significance to industry, providing a review of past relevant research and how this project will build upon it, and describing the 'current climate' of benchtop NIR spectrometry in the Australian sugarcane industry at the commencement of this project in a baseline evaluation.

1.1. Project rationale

This project aims to deliver a technology that can improve the cost and profitability of the Australian sugar industry by allowing more responsive and efficient milling practice, potentially resulting in increased sugar production.

Sugar factories experience significant variation in cane quality across grower consignments. This is managed through optimised control of factory unit settings, to minimise the impact on overall efficiency. Traditionally, the apposite changes to factory settings respond to analytic feedback from mill laboratories that, due to method complexity and limited resources, can be delayed significantly (4 - 24 hours). Consequently, production staff are forced to react to process changes on a limited shift- or daily-basis.

The provision of rapid, low cost analytical data to production staff and process operators will improve process efficiencies in the following areas:

- Milling train extraction- by measuring the pol and moisture variation in final mill bagasse for adjustment of wash water and mill speed.
- Mill mud processing- by measuring pol, moisture and fibre in mud for adjustment of rotary filter settings, bagacillo addition and mud processing rates.
- Pan stage crystallisation- by measuring variation in syrup, molasses and massecuite composition to optimise boiling formulas (B molasses boil-back) and achieve maximum possible crystal content in massecuites. As well as, early detection of impurities that adversely affect crystallisation.
- Low-grade sugar recovery- by measuring variation in C massecuite and magma to optimise low-grade centrifuge settings, minimising final molasses purity and quantity, as well as, early detection of magma impurities that adversely affect crystallisation.
- Raw sugar production- by measuring the pol and moisture variation in raw sugar to optimise the high-grade centrifuge and sugar-drier settings, allowing sugar-quality requirements to be met (attracting bonus payments and avoiding penalties).

Near infrared (NIR) spectroscopy is used as a rapid analytic alternative to traditional methods in many industries as it requires minimal sample preparation, can be performed by inexperienced staff, and will qualify or quantify multiple analytes in less than one minute. Analysis of sugar factory products by NIR spectroscopy has been undertaken previously in many cane-growing countries. However, investigations are often limited to single seasons, mills, products and analytes, which affect the robustness and usefulness of the calibrations. Further, the chemometric approaches to calibration development are often basic and limited by their lack of specificity and tailoring to the product or analyte of interest. Consequently, adoption of laboratory NIR systems is low as calibration quality targets have not yet been achieved.

This project was developed based on the hypothesis that NIR spectroscopic applications in the mill laboratory could be improved by more in-depth analysis of the spectral data and calibration algorithms. This would be achieved by (a) characterising factory products and using the elucidated information and resourceful chemometrics to develop new, molecularly-targeted NIR calibrations, (b) development and validation of calibrations on newly-available, laboratory-appropriate diode array NIR instrumentation, and (c) studying and explaining the causal chemical relationships behind the NIR calibrations to provide evidence-based reasoning for the success of the models. Ultimately, the project outputs should enhance the profitability of the industry by developing rapid, cost-effective analytical solutions.

The key products of interest and their constituents were identified in the early stages of the project through consultation with mill Engineers and are summarised in Table 2. During these discussions, it became evident that NIR spectroscopic analysis provides economic benefit along two separate lines. First, it can save money by increasing the frequency and number of analyses, therefore allowing better refinement of the process stream. Second, it can be useful for replacing reference methods that are time- or labour-intensive, also reducing costs. Value was primarily measured by the benefit of more frequent analyses and better process control (improved recovery), however some also saw value in potential de-manning. For a number of years, the Australian sugarcane industry has been driven by cost savings (often resulting in de-manning) and as a result, gains in revenue have potentially been lost. The advent of cost-effective, process-driven instrumentation (<$80,000), will hopefully strike a balance between capital outlay and increase in revenue through improved recovery.

Raw sugar was consistently identified as the key product of interest, with quality parameters affecting sugar marketability and financial gain through premium bonuses. Millers operate in a high-cost environment and when the sugar price drops, customers are more demanding of quality, but laboratory staff are often the first to be lost when economising. Currently, an Australian mill (that doesn't operate its laboratory 24 hours) cannot correct deviations from the premium sample range due to the slow turn-around time of the laboratory analysis (approximately 5.5 hours for moisture). Therefore, they rely heavily on the skill and judgement of their operators. A fully commissioned NIR analyser could provide immediate results around the clock, allowing raw sugar quality targets to be met through process adjustment, providing the double benefit of maintaining a reputation for high-quality sugar in addition to the accrual of financial bonuses.

Molasses was another product of considerable interest. Final molasses is the highest loss area in a sugar factory. A miller described a scenario where a financial gain of $150,000 could be made with an easily achievable 0.1% improvement in recovery (based on projections for their 2014 crop and a sugar price of $420/tonne). Particular scenarios where analysis of molasses by NIR would reduce final molasses losses are: (a) more frequent analysis of the centrifugal operation would provide intelligence on the station performance and enable responsive process adjustments (molasses is typically analysed on a station/shift basis). Additionally, rapid analysis tools would encourage individual fugal outputs to be measured; identifying fugal-specific issues potentially masked by common station results, (b) lower molasses losses could be incurred by monitoring the A and B molasses and optimising the product presented to the low grade fugals, (c) evaluation of the low grade fugal performance by more frequent analysis of the purity measures in magma, and (d) analysis of the mother liquor properties in massecuite will provide information on the exhaustion of sucrose across the pan stage and allow adjustments to be made to improve the overall or pan-specific efficiencies.

Strong returns can also be expected by utilising NIR spectroscopy for milling train analyses. Measurement of pol and moisture in final mill bagasse suffers due to poor sampling reliability. Basic

statistics describes significant improvement in sampling simply by increasing the number of analyses. This is easily achieved with the assistance of NIR spectroscopic methods. Measurement of these parameters will allow better control of maceration across the milling train. Analyses for pol in open cells (POC) will also benefit from improved sample representation by increased throughput. Benefits would also accrue by using NIR spectroscopic methods for analyses of prepared cane to allow fibre to be accounted for in the crush budget and ensure the cane is at ideal maturity.

Mill mud is another stage where sugar losses can be plentiful if the filter process is not carefully controlled. However, the traditional mud pol methods are relatively quick in their own right so its priority as an NIR application is less.

Table 1: Key products and their major constituents of interest

Raw sugar	Molasses	Massecuite[1]	Bagasse	Cane	Juice[2]	Mill mud
Moisture	Brix	Brix	Pol	Fibre	Brix	Pol
Pol	Pol	Pol	Moisture	Maturity	Pol	Fibre
Ash	DS	DS	Fibre	POC	CCS	Moisture
Colour	Sucrose	Sucrose	Ash	Brix	Ash	
RS	RS	CP		Moisture		
	Ash					

[1] includes magma: [2] includes syrup; RS: reducing sugars; DS: dry substance; CP: cyclone purity; POC: pol in open cells; CCS: commercial cane sugar

1.2. Review of near infrared spectroscopy in the sugarcane industry

A relatively young technique, the use of NIR spectroscopic methods to measure various parameters in agricultural products began in the 1960s with Karl Norris in the wheat industry, who used it to measure protein, moisture and hardness in grains (Hart et al., 1962, Massie and Norris, 1964, Norris and Hart, 1965). Data was collected on simple fixed filter instruments and calibrations were developed using basic techniques such as multiple linear regression (MLR). Rapid, parallel advancements in instrumentation and chemometrics, the multivariate statistical methods for calibrating the instruments, saw the technique spread quickly to various, other branches of agriculture. Soon, NIR spectroscopic methods were being used to measure nutrient contents in forages (Clark et al., 1987, Norris et al., 1976) and vegetables (Halgerson et al., 2004, Villatoro-Pulido et al., 2012), quality and ripeness parameters of fruits (Niu et al., 2014, Pissard et al., 2013, Huang et al., 2008), soil health parameters (Chang et al., 2001, Couillard, 1996, Cozzolino and Moron, 2006, Du and Zhou, 2009) and even constituents of animal meat (Huang et al., 2008, Geladi et al., 1985) and wastes (Huang et al., 2007, Reeves, 2001, Malley and Vandenbyllaardt, 1999, Reeves and Van Kessel, 2000), among other things. Over time, analysis progressed from dried and ground samples to unprepared, raw samples, which facilitated rapid feedback in a process environment.

The global sugar industry began investigating NIR spectroscopy as an analytical technique in the late ‘80s and early ‘90s and was driven mostly by research out of South Africa and Australia. Much of the early work used fixed filter instruments to look at prepared cane (Schaffler and Meyer, 1996) and juice (Meyer and Wood, 1988) samples for payment purposes. Initially, this work did not produce calibrations capable of replacing the reference techniques, as reported by Meyer and Wood, who investigated the application of filter-based NIR systems to measure brix, pol and sucrose in juice and brix, pol, purity, dry matter and fibre in prepared cane. The juice calibrations were far superior to the cane calibrations. Their results were comparable with similar analyses completed by Sverzut et al. (Sverzut et al., 1987).

Soon after, Australian researchers investigating the measurement of similar parameters in prepared cane and pressed juice for clonal performance evaluation identified major problems with sample presentation of cane increasing the errors of the technique (Berding et al., 1991).

In 1989, Ames et al. reported the first use of a scanning instrument in the sugarcane industry, which was said to improve the accuracy of the technique. They developed calibrations for eight quality parameters in Australian raw sugar, including: pol, moisture, reducing sugars, ash, colour, fines, crystal size and crystal elongation. Although, continuing to use univariate regression techniques, they didn't find much gain in using the extended range of the scanning instrument and like the previous studies, were not able to achieve accuracies that allowed the use of NIR analysis for payment purposes. However, they did report acceptable results for use in factory control purposes (Ames et al., 1989b). Clarke et al. (Clarke et al., 1992) followed this up by investigating the application of a scanning instrument for predicting pol, brix and purity in unfiltered cane juice by linear least squares regression methods. They identified that calibrations for pol and brix were accurate and reproducible, but showed a bias when analysing samples for a different geographic location. They did not specifically mention if analysis by NIR was suitable to replace their existing techniques.

In 1993 both Pax (Pax, 1993) and Schaffler (Schaffler et al., 1993) report calibrations on molasses and both applied multivariate regression technique partial least squares (PLS) regression, which capitalises on the improved range of the scanning instruments. Schaffler reported far superior calibrations with PLS regression models for fructose, glucose, sucrose, pol, brix, dry solids and sulphated ash than with multiple linear regression (MLR). Alternatively Pax reported better performance for sucrose by MLR, but PLS outperformed the alternative for dry substance. Again, neither party achieved results with low enough errors to replace the conventional testing, but reported factory control was viable with monitoring of the bias.

Progressive advances in the techniques included improved sample preparation for cane (Schaffler and Meyer, 1996, Clarke et al., 1994, Clarke et al., 1995, Berding and Brotherton, 1994), but practical results were not achieved until Schaffler and Meyer reported success for pol, brix and dry matter in shredded cane suitable for use in plant breeding and agronomy variety trials (Schaffler and Meyer, 1993) and Berding and Brotherton reported success in measuring brix, CCS, fibre, moisture and pol in fibrated cane (Berding, 1995). Soon after the latter moved the technology from the laboratory to an at-line system called SpectraCane (Brotherton and Berding, 1998). The same authors provided a comprehensive study of NIR for the analysis of many constituents in prepared cane, bagasse and mill-prepared cane in preparation for a transition to online analysis. The impact of sampling and representation was the focus of the study and the importance of monitoring and ongoing validation of calibrations was highlighted. This work culminated in the advent of online analysis of sugarcane fibre as it passes through the number one mill in 1999. This, termed the Cane Analysis System (CAS), was accurate enough to allow cane payment and is used extensively throughout the Australian sugarcane industry for both payment and process control today. It uses a scanning monochromator system equipped with PLS equations to predict parameters such as fibre, ash, dry matter, pol in open cells, nitrogen in juice, phosphate in juice, potassium, calcium, silica, magnesium, insoluble ash, pol, brix and CCS (Staunton et al., 1999). Soon after, Madsen II et al. reported success with an at-line analyser, the InfraCana, which measures pol, brix, fibre, moisture and ash in cane and is suitable to replace wet disintegration and core press methods for cane payment (Madsen II et al., 2003). In a slightly different, but related approach, Johnson reported success in direct analysis of juice by NIR using modified partial least squares (MPLS) regression models for pol and brix.

This was a laboratory based analysis but had low enough errors that it was acceptable for use in cane payment (Johnson, 2001).

The success of the CAS encouraged the research team at BSES (now SRA) to advance the work of Ames et al. (Ames et al., 1989a) and Brotherton and Berding (Brotherton and Berding, 1995) on raw sugar. Ames et al. developments were described earlier. Brotherton and Berding improved the raw sugar calibration models by applying the multivariate PLS regression technique, which allowed laboratory-based models to be developed for Brand 1 and JA sugars for the following parameters: ash, fines, reducing sugars, colour, moisture, filterability and pol. The errors for moisture and pol were lower than those reported by Ames et al. The sugar analysis system (SAS) was developed by Bevin et al. (Bevin et al., 2002), who applied the CAS hardware to the sugar belt for on-line analysis of quality parameters. The analyser provided predictions for pol, moisture, ash, colour, conductivity ash, filtrability and grist (fine grain, mean aperture and coefficient of variation). The system provided accuracy and reproducibility suitable for process control, payment and storage management for Brand 1 sugar. The authors expected that expanding the calibration to better represent brands JA, IHP and QHP would further improve the applicability of the calibrations. The SAS was enhanced even further by the same team, when calibrations for specific health-related phytochemicals were calibrated for, allowing production of a regulated and controlled, low-GI sugar product (Kannar et al., 2010).

When investigating applications of NIR in the sugarcane industry, the focus was often on the juice and prepared cane, as well as raw sugar quality parameters. This is due to their high value as payment techniques. However, value also exists in the use of NIR spectroscopic methods for process control purposes in the factory to reduce sugar losses and optimise performance. Particular products of interest are bagasse, juice, syrup, massecuites and magma, molasses and mud. Some preliminary work on molasses was reported earlier.

The milling train is a good example of a potential high-loss stage in sugar milling. Maceration rates are controlled by the mill engineers and in the past, were based on daily average fibre rates, which were at least 24 hours out of date. In 2012, Lloyd et al. reported success in controlling their maceration rates by combining the NIR predicted fibre value, provided by a CAS, with data from their tracking system and factory distributed control system. In doing this they achieved tighter control of their maceration rates, which resulted in a more consistent ESJ brix, reducing the operational load on the evaporator stage (Lloyd et al., 2010). A similar outcome can be achieved with the use of a bagasse analysis system (BAS), developed by the same Australian researchers that produced the CAS and SAS analysers. The BAS is an online analyser that sits in the exit chute of the number 5 mill and measures pol, fibre, dry matter, ash, gross calorific value and net calorific value (Staunton and Wardrop, 2006). As a standalone system, this provides value in identifying boiler feedstock that has high calorific value and low ash (good feedstock) and vice versa (poor feedstock), allowing better management of the waste and preventing boiler fouling. When combined with a CAS, the system can be used to provide valuable information on milling train extraction and maceration rate control.

Recently, there has been a global push in research surrounding renewable energy and sugarcane bagasse was identified as an excellent lignocellulosic feedstock for second generation biofuels. When processing lignocellulosic feedstocks for either thermochemical or biochemical pathways, it is important to understand the composition of the material so the biofuel production can be optimised. The main components of sugarcane bagasse are moisture, ash, extractable matter, lignin (soluble and insoluble), cellulose and hemicellulose. Typically, a good feedstock for biofuels is low in lignin and higher in cellulose and hemicellulose, and also contains low levels of ash.

Researchers at SRA have extensively published their success in developing calibrations for the abovementioned parameters in bagasse and prepared cane (Fong Chong et al., 2013, Oxley et al., 2012, O'Shea et al., 2013a, Chong and O'Shea, 2012), as well as preliminary calibrations for

p-coumaric and ferulic acids in bagasse, which relate to the cross-linking between lignin and the structural carbohydrates (Oxley et al., 2012). The calibrations can be combined with the BAS technology, which can be used to characterise the waste leaving the final mill and direct it for use as either second generation biofuel feedstock (low lignin, low ash), boiler feedstock (high lignin and calorific value, low ash), or alternative disposal such a mulch for high ash material that would cause fouling in the other pathways. Additionally, these calibrations could be combined with the SpectraCane or InfraCana at-line systems to measure these properties in prepared cane for plant breeding purposes. Since, some additional research has been undertaken in this area investigating NIR calibrations for specific anatomical parts of the cane (Sabatier et al., 2012), as well as NIR characterisation of pre-treated bagasse in preparation for biofuel production (Rodríguez-Zúñiga et al., 2014).

Similarly to the early work, much of the research relating to application of NIR spectroscopy for factory control came out of South Africa and Australia. In 1997, Schaffler and De Gaye developed calibrations for pol, brix, conductivity ash, fructose, glucose and sucrose in mixed juice and pol, brix, dry solids, conductivity ash, fructose, glucose, sucrose and total purity difference in final molasses (Schaffler and De Gaye, 1997). This study intended to address the issues raised by Pax (Pax, 1993) and Day-Lewis (Day-Lewis, 1994) about the need for large calibration sets that represent the expected variation in the sample. Schaffler and De Gaye built calibrations on over 550 mixed juice samples and over 900 C molasses samples. Spectral data was collected on a monochromator instrument in transmission mode for mixed juice and reflectance mode for molasses. MPLS regression was used. High-quality calibrations were feasible for sucrose, pol and brix in juice and brix and dry solids in molasses. Average models were achieved for the remaining parameters (Schaffler and De Gaye, 1997). Similarly, Simpson and Oxley analysed the same parameters in the same materials on a Bruker MPA, which is a Fourier-transform (FT)-based laboratory NIR system, which was proposed to be more robust than the existing monochromator systems. Improved SEP vales were reported for all constituents in molasses except for ash and dry solids and all constituents in juice (Simpson and Oxley, 2008). This method was then implemented for routine use in the South African industry (Simpson and Naidoo, 2010a).

In 2010 Simpson and Naidoo released two reports, which both describe calibration development for clear juice, syrups, B and C massecuites, and, A and B molasses samples. As with the previous work, all molasses, syrup and massecuite samples were diluted to allow transmission NIR analysis through a flow cell. One reports calibrations developed on samples from a single season and predicted only pol, brix and sucrose (Simpson and Naidoo, 2010a). Significant improvements in SEP for these parameters were reported in the second article, which reported calibration built with the preceding three seasons data (Simpson and Naidoo, 2010b). In both cases, the results indicated strong predictive performance by the NIR spectroscopic technique and the errors were similar to that of the laboratory. Unfortunately, however, their described application of this technology makes the gains in rapid analysis redundant. To maintain the integrity of the calibrations, they proposed that mills with access to an NIR instrument analyse their samples on-site then send the spectral data to SMRI for processing and the predicted results would be returned to the mill via email, preventing an instant result.

In Australia, researchers at SRA were also investigating the application of benchtop NIR systems for factory products.

In 2011 O'Shea et al. reported successful calibrations for pol, colour, reducing sugars, ash and moisture in raw sugar and sucrose and dry substance in A, B and F and terminal molasses.
The preliminary study used only cross validation to determine potential performance of two different NIR systems (a FOSS dispersive InfraXact™ and a Bruker FT MPA). The raw sugar

calibrations were developed on large, global datasets (minimum n=1238) and the results indicated that evenly matched calibrations could be developed for all constituents on both instruments. The molasses calibrations were developed on samples from a single mill and season and were much smaller (n=56 for MPA and n=119 for InfraXact™). The samples were measured in reflectance mode by the NIR analysers, removing the need to dilute the samples, as the South Africans previously reported.

This would improve the speed of analysis, reduce potential errors and increase the opportunities for online analysis of the molasses stream. The calibrations showed SECV values that were in some cases almost double that of the SEC values, which indicated that overfitting may be occurring. Regardless, the results identify the potential for NIR analysis of raw molasses samples and raw sugar (O'Shea et al., 2011). In 2013 the same authors reported significant advancements in calibration development for a FOSS InfraXact™ instrument in a sugarcane mill and refinery. The comprehensive calibration suite is outlined in Table 2.

Table 2: Calibration suite for the FOSS InfraXact™ (Donald et al., 2013, O'SHEA et al., 2014)

Source	Substrate	Calibrations
Sugar mill	Raw sugar	Moisture, pol
	Juice[1]	Brix, pol
	Mill mud	Moisture, fibre, pol
	Bagasse (Mills 1 - 5)	Moisture, pol
	Pan products[2]	Brix, pol
Sugar refinery	Raw wash	Brix, pol
	Refinery scum	Brix, pol
	Liquors	Colour, brix, reducing sugars
	Syrups	Ash, pol, brix, reducing sugars

[1]Juice includes first expressed juice, mixed juice, evaporator supply juice, juice from mills 1-5 and syrup; [2]Pan products includes A, B, F molasses, A, B, C massecuite

All samples were scanned in their raw form (not diluted) in reflectance mode. Visually transparent samples such as liquor, raw wash, juice, syrup and scum were scanned with the aid of a gold reflector to provide a transflectance scan with 0.4 mm path length. Modified PLS calibrations were developed for all constituents and again, cross validation was used to evaluate the calibration performance of the relatively small datasets (n= 66-457). The results for all calibrations were encouraging as starter calibrations and the mill was able to halve the number of samples subjected to traditional analyses. The authors planned to continue the development of the calibrations and soon after, the system was implemented for routine analysis at Mill 5 and Refinery.

This system provides predicted values for each of the constituents of a product immediately and they are displayed on a screen attached to the instrument. With this instrumentation, there is no need for additional treatment of the spectral data off-site and it exploits the rapid analysis of NIR instrumentation, giving the user immediate information, while protecting the functionality of the calibrations (Donald et al., 2013, O'SHEA et al., 2014).

Some other applications of NIR spectroscopy in the sugarcane industry, not related to factory control and optimisation include analysis of nutrient in leaf tissue (Meyer and Keeping, 2001, Meyer, 1997, Chen et al., 2002, Larrahondo et al., 2001, Keeffe et al., 2015) and mill mud (Keeffe et al., 2014, Keeffe et al., 2013, O'SHEA et al., 2014, O'Shea et al., 2013b), predicting sugarcane pest resistance and clonal performance (Rutherford and van Staden, 1996, Purcell et al., 2005a, Purcell et al., 2005b, Purcell et al., 2007, Purcell et al., 2009, Purcell et al., 2010, Berding et al., 1991), and evaluation of juice and plant stalk in the field to test for maturity (Nawi et al., 2014, Nawi et al., 2013, Nawi et al., 2012).

Overall, NIR spectroscopy is used extensively in the global sugarcane industry. In fact, it is suspected that much of the routine applications are not reported in the literature. NIR spectroscopic analyses are now routinely used for cane payment through analysis of juice and prepared cane. Some preliminary work has also been reported on its application to factory products to improve process control. However, all of the reported work uses FT and monochromator NIR systems, which while fairly robust, require kid-gloves and regular maintenance in a factory environment. The new solid state diode array systems now available are much more cost effective and are designed for harsh factory environments. They are vibration and temperature resistant, dust-proof and have no moving parts. It's expected that these instruments will provide significant value to sugar factories, by allowing rapid analysis right on the factory floor.

1.3. Baseline evaluation

This section provides a brief overview of the current status of benchtop NIR spectroscopic systems in the Australian sugar industry and previous research specifically related to this project. This (along with the performance evaluation in Section 3.4), provides a baseline against which project outputs and outcomes can be measured.

Laboratory-based NIR systems are in frequent use in the global sugar industry. Typically, analysis of juice for brix and pol is performed by monochromator instruments. The Australian sugar industry supports two such instruments, one in a factory setting and one in a refinery setting. Each measures multiple constituents across a variety of factory and refinery products. Alternatively, there is currently no literature describing the use of more stable and factory-appropriate diode array instruments for the analysis of sugar factory and refinery products, nor the application of chemically-targeted chemometric approaches, beyond the basic wavelength section for multiple linear regression methods. However, Tully Mill's recent purchase of a DA1650, which with minor local development, will be capable of raw sugar analyses only (not including fresh raw sugar), illustrates the industry support for development of this technology and the expectation of significant production improvements and cost efficiencies.

Current methods of analysis require sampling, sample preparation and wet chemistry with specialised equipment and trained staff, who suffer frequent exposure to dangerous chemicals. The NIR systems will allow more analyses to be completed with the same resources in a safer environment. Alternatively, human resources could be reduced while maintaining sample throughput.

2. PROJECT OBJECTIVES

This project will improve production efficiencies in the sugar factory by augmenting mill laboratory feedback capacity and allowing proactive adjustment to milling train variations. Specifically, this will be achieved by:

a) Completing full chemical characterisation of sugar factory products to measure the specific molecular activity driving the spectroscopic response and subsequent NIR calibrations.

b) Undertaking time-delay NIR spectroscopic analyses of fresh raw sugar to monitor the reflectance changes observed over time. In combination with chemical characterisation analyses, an assessment will be made on the cause and management of such effects in raw sugar and other sugar factory products.

c) Comparing the benefits of molecularly-targeted NIR calibrations over those developed by the standard, non-specific approach.

d) Assessing the viability and performance of new, diode array bench top NIR instruments and their ongoing stability and maintenance requirements.

3. OUTPUTS, OUTCOMES AND IMPLICATIONS

This section describes the impact that this project has on the Australian sugarcane industry. It is divided into outputs, which describe the products, services or results produced during the project; the outcomes, which are the effects or change realised from successful delivery of the project outputs; and the industry value, which is the benefit to the industry, economy, environment and/or society as a result of realised outcomes. Section 3.4 provides a performance evaluation, against which the success of the project can be measured. Each criterion has been achieved and the relevant section of the report is identified.

3.1. Outputs

This project has delivered a product capable of improving industry profitability and productivity through gains in milling efficiency. Specifically, this project has delivered the following outputs:

a) Product — a calibration suite for sugarcane factory products that provides turn-key or near turn-key DA1650 NIR spectroscopic solutions for mill laboratories. The system is capable of real-time, multi-analyte analysis of the nominated products.
b) Soft technology – a calibration model capable of analysing fresh raw sugar as-is, providing a forward prediction of the stabilised pol and moisture values.
c) Product — an information package to illustrate, scientifically, how and why NIR analysis works for quantifying analytes in nominated sugar factory products. This aims to promote the benefits of the technology and drive adoption across the board.
d) Soft technology — a guideline for users and SRA-NIR Team staff outlining the validation demands of each platform.
e) Soft technology — a standardisation protocol for FOSS DA1650 spectroscopic instruments
f) Communicator/capacity builder — Promotion of the technology through mill trials and demonstration.
g) Tool/enabler – a large database of NIR spectra and matching wet chemistry data.

It was originally proposed that two products would be developed in the form of calibration suites for the FOSS DA1650 and the Perten DA7250.

Unfortunately, the sample presentation issues faced with the Perten DA 7250 rendered it unsuitable for the analysis of sugarcane factory products and therefore, its development was ceased.

The target audience for the product are the mill Production Managers, who are responsible for factory operations and oversight of the mill laboratories. They, along with the Production Engineers benefit from the increased information flow provided by the systems. The Laboratory Analysts and Production Engineers are typically the users of the system. Additional beneficiaries of the system include bulk sugar handlers, sugar refiners and bulk sugar testing laboratories.

To date, the adoption of the system has been relatively low, however the development of the calibration suite during this project has allowed Tully Mill to integrate their system into their routine operations. Additionally, Queensland Sugar Limited (QSL) have purchased and implemented a system for routine use. Several other mills are supportive of purchasing a system, but the timing is poor due to the extensive, industry-wide CAPEX replacement required for the Cane Analysis Systems (CAS).

The extension activities conducted during this project have generated good interest by industry, and the technology has been closely monitored by several milling groups. Promotion and extension activities must continue beyond the project completion in order to capitalise on the momentum generated during this project. This will be conducted through the commercial SRA NIR spectroscopy team, based in Meringa under the Milling Efficiency and Technology banner.

3.2. Outcomes and Implications

The key outcomes that will result from this project speak to each of the key impacts for KFA 5, identified in SRA's strategic plan.

a) Increased/ improved mill laboratory output (Profitability)

NIR spectroscopic analysis will allow real-time, multi-analyte analysis of factory products, with only 10 % of samples requiring analysis by traditional wet chemistry, which will have a significant impact on profitability. More samples to pass through the laboratory for the same number of staff, maintaining throughput with fewer people, or a combination of both. Essentially, the cost-per-sample is reduced.

b) Improved efficiency in the mill (Sustainability)

Improving the dynamic response of the mill to more closely mimic the input/ process variable will improve the sustainability of the mill through minor to intermediate improvements in processing across all stages. In particular, improved fibre rate control of the milling train, as well as optimised maceration rates to balance sucrose extraction and final mill bagasse moisture. At the pan stage, the sucrose/ impurity balance can be monitored. Closer monitoring of these processes will minimise wasted energy use, improve boiler efficiency and allow coordination and optimisation of maintenance in the pan stage. Additionally, feedback on fibre quality and cane maturity can be given to growers and harvester contractors to assist in improving the cane supply quality.

c) More sugar (Profitability)

Implementation of NIR spectroscopic systems in mill laboratories will provide mill production staff access to accurate and timely process data, allowing them to make inter- and intra-stage refinements to minimise sugar losses and improve stage performance and efficiency.

In particular, improvements can be made to minimise sucrose loss through the final mill bagasse, final molasses and mud through pol and sucrose monitoring.

d) Improved sugar quality (Sustainability and Profitability)

More frequent monitoring of the final raw sugar allows optimisation of the fugal stage to meet quality targets, increasing bonus payment frequency and minimising quality give-away. This is particularly valuable in the new marketing climate for the Australian sugar industry, where quality targets for trade may be strictly enforced at the mill.

e) Improved safety (Sustainability)

Many of the analytical methods in the mill require frequent exposure to lead, phenol and other hazardous chemicals. Replacing the majority of these analyses with NIR will limit this to infrequent exposure, reducing risk to the health of the staff and limiting the risk to the milling company.

(f) Support for transition to true purity (Capability, Profitability and Sustainability)

For most products, the benchtop analysis system provides predicted results for both apparent (brix and pol) purity and true (sucrose and dry substance) purity analytes. Where mills still operate under apparent purities, having both sets of results available for all products will familiarise engineers with the true purity comparisons under their standard operating conditions. Additionally, the reduction in analysis frequency may allow mills to transition to the more time-consuming, but more accurate true purity methods for the 10 % validation samples.

(g) Strong collaborative networks (Organisational excellence)

The design of this project resulted in strong industry linkages and collaborations being developed, which facilitated the development of tailored and appropriate systems for the mills.

To date, four systems are in use by the Australian sugar industry, with another mill pursuing purchase and installation. This exceptional outcome directly reflects the milling and marketing community's acceptance of this technology as fit-for-purpose, in a variety of applications. Dissemination of this report and the results enclosed, along with continuing extension activities, will provide additional confidence in the technology and likely result in further adoption.

3.3. Industry value

It is difficult to quantify the specific financial benefit of the technology to the Australian industry; however, potential benefits of this project can be expected in two different areas, milling train extraction and sugar recovery.

Assuming a 10 % adoption rate at 30 million tonnes of cane per year, a conservative 1 % increase in milling train extraction (from 95 % to 96 %), could provide a potential economic benefit of $1.62M per year. SRA online NIR spectroscopic systems for bagasse (BAS) have demonstrated a 2 % increase in milling train extraction (Staunton and Wardrop, 2006, O'Shea et al., 2010), so a 1 % increase from semi-regular monitoring afforded by a benchtop system is not unachievable. Comparably, increasing sugar recovery from 90 % to 91 % under the same conditions could provide a further $1.71M per year.

Milling Train Extraction
Assumptions:

- 1 Million Tonnes of cane
- Pol % Cane = 15
- Sugar Recovery = 90 %
- Sugar Price = $400/tonne

Potential economic benefit from increasing milling train extraction from 95 % to 96 %:

Tonnes sugar in cane supply	= 1,000,000 x (15/100) = 150,000 tonnes
Potential extra sugar recovered by milling train	= 150,000 x (1/100) = 1,500 tonnes
Potential extra raw sugar	= 1,500 x (90/100) = 1,350 tonnes
Potential Economic Benefit	= 1,350 x $400 = $540,000 x 30 = $16,200,000 per annum
At 10 % adoption	= $16,200,000/10 = $1,620,000

Sugar Recovery:

Assumptions:

- 1 Million Tonnes of cane
- Pol % Cane = 15
- Milling Extraction = 95 %
- Sugar Price = $400/tonne.

Potential economic benefit from increasing sugar recovery from 90 to 91%:

Tonnes sugar in cane supply	= 1,000,000 x (15/100) = 150,000 tonnes
Sugar recovered by milling train	= 150,000 x (95/100) = 142,500 tonnes
Potential extra raw sugar	= 142,500 x (1/100) = 1,425 tonnes
Potential Economic Benefit	= 1,425 x $400 = $570,000 x 3 =$1,710,000

Additionally, implementation of NIR spectroscopic methods for routine analysis will reduce the exposure of laboratory staff to myriad dangerous chemicals including lead, phenol and concentrated acids. A reduction in hazardous waste will also result, providing an environmental benefit.

3.4. Performance evaluation

Ultimately, adoption and commercial uptake of NIR systems and calibrations will be the key measure of success for this project. However, the following activities were outlined during the development of the project as outputs against which the performance of the project could be measured. Their completion was to be indicative of project success.

With the exception of activity (? (for which an alternative approach was achieved) all activities have been achieved in full, and are reported in the subsequent sections of this report.

- Attainment of high-quality (within factory processing requirements), molecularly targeted diode array calibrations for nominated products (Section 6.2.2).
- Developed understanding of the reactions occurring in fresh raw sugar and implementation of an analysis strategy for the product (Section 6.2.3).
- Completion of a *Guide to laboratory NIR* to explain the mechanics of the calibrations as well as outline the operation, performance and maintenance expectations of diode array instrumentation and their associate calibrations (Section 4 and Section 6.3).
- Periodic consultation with ASMC Technical Committee to ensure all real-world factors are being considered during calibration development, assessment of economic and productivity value and definition of quality and performance targets (Section 4).
- Demonstration of the reduced analytical cost/unit as well as the reduced environmental cost/unit to define economic and environmental cost advantages (Section 3.3).
- Extensive in-mill instrument trials to demonstrate hands-on the benefits and advantages of such technologies (Section 6.1).
- Publication of project successes in Australian and international conference proceedings and journals (Section 4 and Section 7).

The completion of these activities contributes to strategic SRA RD&A priorities, as outlined in KFA5 of SRA's Strategic Plan 2012/18 - 2021/22. Specifically, the improvement of production efficiency and profitability, through development of efficient data systems, product and process improvement opportunities and identification of barriers to feed supply and factory efficiency; improving the environmental sustainability and energy efficiency, through maintenance of cost-efficient operations; and enhancing knowledge transfer and capability (by providing developing a tool that facilitates training for new and existing industry participants). As identified in Sections 3.2 and 3.3, these project activities have contributed to the Strategic Plan key impacts for improving profitability and sustainability in the Australian sugarcane industry and capability and organisational excellence within Australian Sugarcane research.

4. INDUSTRY COMMUNICATION AND ENGAGEMENT

4.1. Industry engagement during course of project

This project has maintained strong industry engagement throughout its operation, beginning at the planning phase. The structure of the project and selection of the analytes of interest was based on consultation and feedback with millers at Wilmar, Tully Sugar/ COFCO, MSF Sugar, Sunshine Sugar and Heck Group. Once the project began, 12 mills from six milling groups participated in the large-scale sampling conducted in year one. In the following three years, 13 trials were conducted in nine mills, including: Tableland Mill, Mulgrave Mill, Maryborough Mill, Farleigh Mill, Tully Mill, Mossman Mill, Isis Mill, Condong Mill and Victoria Mill. The intention behind the extensive trial work was to give millers hands-on experience of the technology, in their standard operating environment.

On multiple occasions, the system was used to provide supporting data to research activities being conducted by other parties, at the mill.

In addition to the direct contact with the industry through the sampling and mill trial processes, formal project updates were provided through SRA's Regional Research Forums each year and the

ASSCT conference in 2015 and 2016. Outcomes were also reported during the SRA CAS User Group Meetings.

In 2016, a DA 1650 was hosted in the SRA booth at the ASSCT Tradeshow, along with molasses and raw sugar samples. This was an extremely useful demonstration strategy, with mill staff and Engineers from each Australian mill testing it and providing feedback. As a result of this display, an instrument was sold to Queensland Sugar Limited and implemented into their processes within a few weeks (see Section 6.3.1).

Following the completion of this project, the trial work and ongoing development of the technology will continue via the commercial SRA NIR spectroscopy team, based in Meringa. This will be permitted through the commercial support agreements entered into with certain mills.

4.2. Industry communication messages

The key messages derived from this project include:

- Turn-key FOSS DA1650 NIR spectroscopy systems have been developed for analysis of key factory products.
- The precision data associated with the NIR spectroscopic technique is better than the published precision metrics available for comparable Australian and International methods.
- Fresh raw sugar can be directly analysed by NIR spectroscopy due to success with desensitised models.
- Global calibrations, along with FOSS' standardisation procedure, allow direct transfer of models between instruments.
- At this point in time, the sample presentation of the Perten DA7250 NIR spectroscopic instrument do not make it suitable for the analysis of sugarcane factory products.
- Molecularly-targeted calibration models do not improve the performance or selectivity of the calibration models. Rather, they are stabilised by the multi-collinearity of the full wavelength range of the DA1650 instrument.
- An industry report describing the mechanism of NIR spectroscopic analysis of sugarcane factory products is available.

5. METHODOLOGY

This project was broken down into three concurrent activities based around (a) transfer and development of standard models, (b) sample characterisation and molecularly-targeted model development, and (c) platform performance. For clarity, Sections 5 and 6 will be reported against these activities. Section 5 describes the methodologies that were used to conduct the project and/or experimental activities. The subsequent results and discussion for each activity is provided in Section 6.

5.1. Transfer and development of standard NIR spectroscopic calibration models

This section describes the development of NIR spectroscopic calibration models by the standard methods developed by SRA. This includes calibration transfer, calibration development and model validation. Additionally, validation of NIR spectroscopy as an analysis technique has been evaluated.

5.1.1. Large-scale preliminary sampling

Each process stream was sampled at regular intervals, according to the standard operational practice of each mill. Typically this included a mixture of shift, daily and weekly composites for each of the key factory products. For bagasse, and the pan products, where multiple intermediates are generated (e.g. A, B, F Molasses), each were sampled and analysed separately.

Each mill laboratory analysed the samples according to their usual methods and retained any residual sample. Typically, the methods were those described in the Laboratory Manual for Australian Sugar Mills – Volume 2 (2001b). Brix was measured by Method 16 and sucrose by both Method 18 and by high performance liquid chromatography (HPLC), depending on the mill.

The residual sample was frozen and transported to SRA's Brisbane laboratory for analysis by NIR spectroscopy. Samples were maintained in freezer storage upon arrival at SRA.

For analysis by NIR spectroscopy, samples were defrosted in batches at room temperature with minimal lead-time to preserve the integrity of the sample. All samples were mixed thoroughly prior to analysis by NIR spectroscopy to reconstitute any water and sugar syrups. For the solid C massecuite samples, a microwave was used to heat the sample to approximately 60 °C to allow mixing and presentation to the sample cup. All other samples were analysed at room temperature.

Each sample was analysed on a FOSS DA1650 benchtop NIR instrument (FOSS NIRSystems, Hillerød, Denmark), owned by SRA, and a loaned Perten DA 7250 benchtop NIR instrument (Perten Instruments, Hägersten, Sweden). Spectra were also collected on fresh samples using the Tully Mill DA1650, which was located in the mill laboratory.

On the DA1650, the spectra were collected with 0.5 nm spacing across the full wavelength range of the instrument (1100 - 1650 nm). Auto integration was used, with the upper limit switched off for molasses. Raw sugar, molasses and massecuite were analysed using a small cup, juice and syrup were analysed using a small cup and 0.4 cm transflectance accessory and bagasse, prepared cane and mill mud were analysed using a rotating large cup to increase the surface area of the analysis.

On the DA7250, the spectra were collected with 0.5 nm spacing across the full wavelength range of the instrument (950 - 1650 nm). Auto integration was used. Raw sugar was analysed using a disposable cup, massecuites were analysed using a syrup cup, juice and syrup were analysed using a transflectance cell, molasses was analysed using a clear liquids cup and bagasse, prepared cane and mud were analysed using a rotating large sample dish.

5.1.2. Development of transfer calibration models

Near infrared spectra of sugarcane factory products, collected using FOSS InfraXact™ and FOSS DA1650 instruments, along with the associated wet chemistry data, was collated. This data was generated at many different mills on multiple instruments of each type as a result of past instrument trials and the commercial InfraXact™ installation at Mill 5.

The InfraXact™ data was used as the calibration data set, and the calibration models were validated with the all of the DA1650 and DA7250 data available. This included data from past trials, as well as some data from the large-scale sampling conducted in the early stages of this project.

NIR spectroscopic and wet chemistry data was not available for all products and constituents identified by industry in Table 1. Without data, transfer calibrations could not be developed. Table 3 shows the products and constituents for which data was available and transfer models could be developed.

Table 3: Key products and their major constituents of interest

Raw sugar	Molasses	Massecuite[1]	Bagasse	Cane	Juice[2]	Mill mud
Moisture	Brix	Brix	Pol		Brix	Pol
Pol	Pol	Pol	Moisture		Pol	
	DS	DS				
	Sucrose	Sucrose				

[1] includes magma: [2] includes syrup; RS: reducing sugars; DS: dry substance; CP: cyclone purity; POC: pol in open cells; CCS: commercial cane sugar

All calibrations were developed by adapting the traditional SRA methods that are used for the CAS calibration model development. These use wavelength regions and pre-treatments defined in the early days of NIR calibration development at SRA. They were identified to consistently produce robust calibrations, minimising the error related to the high moisture content of sugarcane industry samples.

Models were developed using Unscrambler® X version 10.4 (Camo, Norway). All spectra were treated with a Gap-Segment first derivative (gap 1, segment 6) followed by a standard normal variate (SNV) transform. The spectra were then trimmed to two segments 1100 - 1370 nm, 1410 - 480 nm. Calibrations were developed using partial least squares (PLS) regression. The calibration set included all InfraXact™ data and the validation set included either, all DA1650 data, or all DA7250 data as an independent validation. The DA1650 and DA7250 were validated separately.

Calibration models were evaluated on their standard error of calibration (SEC), standard error of prediction/ validation (SEP), bias, R^2 and number of factors used to develop the model. Parsimonious models were favoured.

5.1.3. Development of standard calibration models

The method for the development of standard calibration models was modified slightly with each calibration update, due to learnings from those previously.

5.1.3.1. Global 15.0

The Global 15.0 models were developed in Unscrambler® 10.3 X, using the spectral treatment and calibration method described in Section 5.1.2.

Calibrations were developed using a dataset containing IX data, DA1650 data and DA7250 data in combination. For each product, a single mill, for a single season (if data existed for more than one season), was excluded from the dataset to be used as the validation set. The data in the validation set was chosen based on sample size and the constituents reported against it.

5.1.3.2. Global 15.1

The Global 15.1 calibrations were developed for implementation onto their respective instruments. The Perten and FOSS instruments are each calibrated using independent software. As much as possible, the conditions were maintained across platforms, however, slight variation in data processing and PLS algorithms can be expected between the two software packages. In essence, the calibration data was treated with a first derivative transform, with a gap of 1 and segment of 4 (or equivalent), followed by a standard normal variate transform. The moisture regions were removed and calibrations built using partial least squares (PLS) regression. Multiple data sources (instruments, seasons, mills) were used to develop global calibrations.

The calibration conditions and models of each platform are described:

FOSS DA1650

- Data source/s: FOSS DA1650, FOSS InfraXact™
- Models: Bagasse and prepared cane, juice and syrup, molasses (A, B, F), massecuite (A, B, C) and magma, mud, raw sugar
- Modelling software: WinISI version 4.11, FOSS Analytical A/S, Denmark.
- Pre-treatment: Derivative and SNV, 1,4,4,1
- Wavelength range: 1100 - 1370 nm, 1410 - 1480 nm
- Data spacing: 0.5 nm
- Regression algorithm: Modified PLS
- Validation: Independent

Perten DA7250

- Data source/s: Perten DA7250, FOSS DA1650. If an excess of DA1650 data was available, random samples were removed until n was more equivalent for each dataset.
- Models: Bagasse and prepared cane, juice and syrup, molasses (A, B, F), massecuite (A, B, C) and magma, raw sugar
- Modelling software: The Unscrambler® X Version 10.3, Camo Software AS, Norway.
- Pre-treatment: Derivative (gap 1, segment 6) SNV.
- Wavelength range: 1100 - 1370 nm, 1410 - 1480 nm
- Data spacing: 0.5 nm
- Regression algorithm: PLS
- Validation: Independent. Cross validation where sample numbers were small

5.1.3.3. Global 15.2

Mid-way through the 2015 crushing season, the DA1650 global calibration models were updated. This update saw the inclusion of a significant amount of historic data from alternative platforms. In particular, additional data was sourced from the FOSS XDS (monochromator) and the FOSS ProFOSS™ (diode array).

Changes to the calibration method include the expansion of the wavelength range to 1100 nm - 1650 nm and use of a 2 nm data spacing. Validation was conducted through independent mill trials.

5.1.3.4. Global 16.1

During the 2016 crushing season, the DA1650 global calibration models were updated with the additional DA1650 data generated during the trial work. Method changes from the Global 15.2 calibration update (Section 5.1.3.3) include the use of 0.5 nm data spacing and PLS regression technique, as opposed to modified PLS. Validation was conducted through independent mill trials.

For the trials at Mill 2, a summary of the expected analyte boundaries for each product was provided.

This was used to evaluate outliers in the wet chemistry data. If there was a large deviation between the NIR predicted value and the wet chemistry value and the wet chemistry value the values were interrogated. Where the NIR predicted value was in range and the wet chemistry value out of range, the sample was removed as an outlier. Where the wet chemistry was within range and the NIR predicted value was outside of the range, the sample was not removed as an outlier. If the wet chemistry result was grossly different due to a clear labelling error, the sample was removed from the analysis.

5.1.3.5. Global 17.1

The calibration dataset for the DA1650 Global 17.1 calibration models was cleaned using Saurkraut, an in-house spectroscopy software package. Samples with high levels of noise observed with a second derivative spectral treatment were removed by hand.

Subsequent calibration development occurred using the same techniques as for the Global 15.2 models (Section 5.1.3.3), and all models were forced to 16 factors. Validation was conducted through independent mill trials.

Outliers for Mill 2 were evaluated in the same manner as Section 5.1.3.4.

5.1.4. Method validation

The following methods were used to evaluate NIR spectroscopy as a method according to analytical method validation criteria.

5.1.4.1. Accuracy

Several experiments were conducted for each product to evaluate the accuracy of the NIR spectroscopic method. They are described in Table 4.

Table 4: Accuracy experiments for sugarcane factory products

	Target analyte	Experiment
Raw sugar	Reducing sugars	Equal proportions of glucose and fructose added to 20g and 40g sugar samples in 0.1 g increments. 3 different sugar samples.
	Ash	Silicon oxide added to 40 g and 50 g raw sugar in 0.1 g increments. 3 different sugar samples.
	Ash	Equal proportions of KCl and $CaCl_2$ added to 40 g of sugar in 0.1 g increments then 0.5 g increment. At high ash loading, dried in oven and analysed at room temperature.
	Colour	200 µL, 500 µL and 1000 µL of cooked fructose added to 50g of sugar
	Moisture	Sample dried for 3hr at 65 degrees. 1mL MQ added to 65 g sugar in 2 increments.
	Matrix	1 g sand added to 65 g sugar
	Sample size	10 g, 20 g and 50 g untreated sugar
	Pol	0.1 - 0.05 g of sucrose added to 50 g raw sugar in 5 increments
A Molasses	Ash	Silicon oxide added to 70 g molasses in two 0.1 g increments.
	Ash	Equal proportions of KCl and $CaCl_2$ added to 70 g of molasses in two 0.1 g increments followed by one 0.5 g increment
	Reducing sugars	Equal proportions of ground glucose and fructose added to molasses samples. 1 - 5 g added in several increments.
	Sucrose	1 – 20 g of ground sucrose added to 70 – 90 g of molasses.
	Purity	Equal proportions of A and B mol mixed. Equal proportions of A and C mol mixed.

	Target analyte	Experiment
B Molasses	Ash	Silicon oxide added to 70g molasses. 1 g, 5 g, 10 g.
	Ash	Equal proportions of KCl and $CaCl_2$ added to 70g of molasses. 0.1 g, 0.2 g and 1 g increments
	Reducing sugars	Equal proportions of glucose and fructose added to 65 g molasses samples. 5 g added.
	Sucrose	10 g sucrose added to 65 g of molasses
	Moisture	MQ added to 65 g molasses. 2 mL, 3 mL, 5 mL increments.
C Molasses	Ash	1g of equal proportions of KCl and $CaCl_2$ added to 64 g of molasses.
	Reducing sugars	Equal proportions of glucose and fructose added to 65 g molasses samples. 5 g added.
	Sucrose	10 g sucrose added to 65 g molasses
	Moisture	5 mL MQ added to 65 g molasses three times.
Bagasse	Moisture	5 mL MQ added to 30 g of bagasse
	Reducing sugars	2 g glucose/fructose mixture added to 25 g bagasse
	Sucrose	2 g sucrose added to 2 5g bagasse
	Moisture	25g oven dried at 50 degrees overnight
	Matrix	Sand added to 25 g of bagasse. 2 g then 5g
Syrup	Ash	0.2 g and 0.5 g of equal proportions of KCl and $CaCl_2$ added to 10 g of liquor
	Reducing sugars	Equal proportions of glucose and fructose added to 10g liquor sample. 0.2 g, 0.5 g, 1 g.
	Sucrose	Sucrose added to 10g liquor. 0.2 g, 0.5 g, 1 g, 2 g.
Juice	Ash	0.2 g, 0.5 g, 1 g of equal proportions of KCl and $CaCl_2$ added to 10 g of juice
	Reducing sugars	Equal proportions of glucose and fructose added to 10g juice sample. 0.2 g, 0.5 g, 1 g.
	Sucrose	Sucrose added to 10 g juice. 0.5 g, 1 g.

5.1.4.2. Precision

To calculate the repeatability and reproducibility of the NIR spectroscopic technique, two series of experiments were conducted. The first aimed to assess the precision of the analysis for a single sample. The second evaluated the method precision for a population.

For the first experiment, the SRA and QSL instruments were installed in a single laboratory. Two analysts each scanned one sample of each product six times, re-packing the sample cup each time. The final re-pack was scanned six times without replacement. The products analysed were: final mill bagasse, A molasses, syrup, mud, prepared cane, raw sugar. The standard deviation of repeatability (S_r) was determined by calculating the square root of the average of the variance of the repeated measures across each lab and operator. The standard deviation of reproducibility (S_R) was determined by calculating the square root of the average of the variance of the replicated measures across each lab and operator. The following equations were then used to obtain the repeatability and reproducibility metrics:

$$repeatability\ (r) = 2S_r$$

$$reproducibility\ (R) = 2S_R$$

$$standard\ uncertainty\ at\ 95\%\ confidence\ interval\ (u) = \frac{R}{\sqrt{n\ contributors}}$$

$$expanded\ uncertainty\ (U) = 2u$$

For the second experiment, a population of raw sugar samples (34) were analysed on the SRA and Tully Mill instruments in succession. Each sample was analysed on each instrument with two repacks, and each repack scanned twice.

The S_r was calculated by averaging the re-scans and determining the standard deviation of the difference between the two repacks for each instrument. The repeatability was calculated by:

$$repeatability\ (r) = 2S_r$$

The S_R was calculated by averaging the re-scans and re-packs for each sample on each instrument and determining the standard deviation of the difference between the two instruments.
The reproducibility was calculated by:

$$reproducibility\ (R) = 2S_R$$

As the set was representative of the population, the u is approximately equal to S_R, and the U was calculated by:

$$expanded\ uncertainty\ (U) = 2u$$

The predicted values used to calculate the precision metrics were from Global 17.1 for the pan products and Global 16.1 for raw sugar bagasse, prepared cane and mud.

5.1.4.3. Specificity

The specificity of the Global 17.1 models was evaluated by the bias (c) and slope (m) of the least squares regression.

$$y = mx + c$$

5.1.4.4. Sensitivity

The sensitivity of the Global 17.1 calibration models was evaluated by determining the standard deviation of the slope of the calibration curve using the following formulae (Harris, 2007):

$$S_m{}^2 = \frac{S_y{}^2 n}{D}$$

where:

$$S_y = \sqrt{\frac{\sum(d_i{}^2)}{n-2}}$$

and:

$$D = \begin{vmatrix} \sum(x_i{}^2) & \sum x_i \\ \sum y_i & n \end{vmatrix}$$

And using this to calculate the smallest difference than can be distinguished between two measures using the following equation (Massart, 1998):

$$d = \left(t_{1-\frac{\alpha}{2}} + t_{1-\beta}\right) S\sqrt{2}\left(\frac{1}{m}\right)$$

5.1.4.5. Linearity

The linearity of the calibration model is based on the least squares regression of the NIR predicted and reference values and the squared correlation coefficient of the regression line.

It is calculated by (Harris, 2007):

$$R^2 = \frac{[\sum(x_i - \bar{x})(y_i - \bar{y})]^2}{\sum(x_i - \bar{x})^2 \sum(y_i - \bar{y})^2}$$

5.1.4.6. Range

The calibration range is based on the distribution of the calibration plot over which the calibration linearity and residuals are maintained.

5.2. Sample characterisation

This section describes the methods used for the extensive analyses of sugarcane factory products by advanced wet chemistry and spectroscopic techniques to characterise the chemical composition of the products. Following characterisation, NIR spectroscopic calibration models were developed using a non-traditional approach.

5.2.1. Characterisation of nominated products

Molasses, bagasse and raw sugar were subjected to high-end chemical and spectroscopic analysis such as thermogravimetric analysis (TGA), infrared (IR) spectroscopy, Raman spectroscopy, ion chromatography and inductively coupled plasma atomic emission spectroscopy (ICP-AES), among others.

5.2.1.1. Molasses

A subset of molasses samples were subjected to further analysis for moisture, dry solids, ash, sugars, major and trace nutrient elements, and carbon. A selection of samples were also analysed by TGA, IR spectroscopy and Raman spectroscopy.

Freeze drying
Samples were weighed into specially designed containers, snap frozen in liquid nitrogen and freeze-dried for 86 hours at 0.09 mbar. Once dry, the samples were very deliquescent and extreme care was required to prevent re-uptake of moisture when re-weighed. The samples were ground using a mortar and pestle and dried for another 24 hours in a vacuum oven at 40 °C.

Ion chromatography
Samples were diluted in water, filtered and analysed on a Dionex ICS-3000 with electrochemical detector. Compounds were separated on a CarboPac™ PA1 column and guard using Milli-Q® and sodium hydroxide as eluents.

ICP-AES
Samples were diluted in water and analysed on an Agilent ICP-AES fitted with a Sturman-Masters double pass spray chamber and V-groove nebuliser.

DUMAS Combustion
Carbon and nitrogen analysis was performed by the Dumas combustion method using an Elementar Cube CN analyser.

FT-IR
Freeze dried molasses was analysed by a Nicolet 5700 FT-IR fitted with an ATR accessory with a diamond interface.

The following instrument conditions were maintained:

- Range: 4000 - 620 cm^{-1}
- Number of scans: 128
- Resolution: 4 cm^{-1}
- Gain: 8
- Velocity: 0.0329 cm/ sec
- Spectral correction: ATR – Diamond (angle 45, bounces 1, RI 1.50)

The sampling interface was cleaned and a new background collected between each sample.

TGA
Two TA Instruments Q500 TGA instruments were used for the analyses of these samples. Samples were analysed using a platinum pan, with nitrogen as the balance and sample gas. The temperature program had a 10 minute isothermal period followed by a ramp at 5 °C/ min to 1000 °C.

Raman spectroscopy
Not feasible due to excessive fluorescence.

5.2.1.2. Raw sugar

A subset of raw sugar samples were subjected to further analysis for moisture, dry solids, ash, sugars, major and trace nutrient elements, and carbon. A selection of samples were also analysed by TGA, IR spectroscopy and Raman spectroscopy.

Drying
A sub-sample was dried in an oven at 100 °C until constant mass.

Ash
To measure ash, samples were subjected to Method 26 of the BSES Laboratory Manual for Australian Sugar Mills Volume 2 (2001a).

Ion chromatography
Samples were prepared and analysed as described in Section 5.2.1.1.

ICP-AES
Samples were prepared and analysed as described in Section 5.2.1.1.

DUMAS Combustion
Samples were prepared and analysed as described in Section 5.2.1.1.

FT-IR
A sub-sample of dried sugar was ground using a mortar and pestle in a refrigerated environment to minimise moisture uptake. The powdered sugar was analysed by FT-IR under the same instrument conditions described in Section 5.2.1.1. Spectra were also collected on whole crystals, but the data are not reported.

Raman spectroscopy
Not feasible due to excessive fluorescence.

5.2.1.3. Bagasse

Extractives
The extractives method uses accelerated solvent extraction with water and ethanol to isolate extractable components, which are solidified and measured by mass.

Ash
Total ash was measured by muffle furnace. Samples were placed in a muffle furnace set to 105 °C. The temperature was slowly increased to 575 °C and left for three hours.

FT-IR
Previously sieved and ground bagasse samples were dried in oven at 105 °C overnight. They were cooled in a desiccator stored in the walk-in-fridge (at approx. 4 °C) for 15 minutes prior to further grinding by mortar and pestle. The ground bagasse was analysed by FT-IR under the same instrument conditions described in Section 5.2.1.1.

5.2.2. Development of molecularly-targeted calibration models

Molecularly-targeted calibration models for molasses were developed in WinISI. The calibration set used for G16.1 was used. The process for developing the calibration model is the same as that described in Section 5.1.3.4, except, instead of the full wavelength range being used, the wavelength ranges defined in Table 5 were used to develop each model.

Table 5: Active NIR spectroscopic regions by analyte

	Wavenumber (cm^{-1})	Wavelength (nm)	Correlation
Sucrose/pol	7300-7200	1370-1390	Negative
	7100-7000	1405-1430	Positive
	6900-6800	1450-1470	Positive
Dry substance/brix	8850-8750	1130-1145	Negative
	8660-8580	1155-1165	Positive
	8400-8250	1190-1215	Positive
	7215-7082	1385-1415	Positive
	7000-6900	1425-1450	Negative
Ash/reducing sugars	8400-8330	1190-1200	Positive
	7250-7200	1380-1390	Positive
	7050-7000	1415-1430	Negative
	6900-6850	1450-1460	Negative
	6570-6520	1520-1535	Positive

5.2.3. Analysis of fresh raw sugar

This section describes the experiments conducted to model the spectral change in fresh raw sugar.

5.2.3.1. Time-delay analysis of fresh raw sugar

Several time-delay analyses similar to the 2013 trials were completed, however, the samples were stored in an oven or refrigerator in between analyses to minimise the impact of temperature variation on the predicted values. The temperature of the actual sample was collected along with the age of the sample at each analysis point. The following experiments were completed in triplicate:

- Experiment 1 – Brand 1 stored in 25 °C oven
- Experiment 2 – JA stored in 23 °C oven
- Experiment 3 – JA stored in 25 °C oven

- Experiment 4 – JA stored in 27 °C oven
- Experiment 5 – JA stored in 30 °C oven
- Experiment 6 – JA stored in 33 °C oven
- Experiment 7 – JA stored in 4 °C refrigerator
- Experiment 8 – JA stored in 23 °C vacuum oven at -40 kPa

For each experiment, three samples were collected in separate buckets. Samples were collected from the sugar belt immediately after exiting the drier and the time and temperature were recorded. Samples were taken to the laboratory, mixed and sub-sampled into a small NIR sample cells. The samples were stored in the appropriate conditions and scanned periodically on the NIR instrument. The time and sample temperature were recorded for each scan point. The Global 15.2 sugar models, described in Section 6.1.3.3, was used to predict the pol and moisture values of each sample at each time point.

5.2.3.2. Modelling fresh raw sugar

Fresh raw sugar models were developed using the standard procedure described in Section 5.1.3.4. However, the calibration dataset was modified to desensitise the calibration model.

First, change curves were developed for 20 samples by scanning them periodically over an extended period of time. For many of the samples, the instrument was installed close to the raw sugar belt to allow analysis from a sample age of 40 seconds. The final scan time varied from raw sugar age of 295 minutes to 1,437 minutes. For each sample, the predicted pol and moisture value for all scans after 200 minutes were averaged. The average predicted value was assigned as the wet chemistry value for each scan for that sample. These spectra and associated ‘wet chemistry’ data form the basis of the fresh raw sugar calibration set.

The remaining 105 samples in the calibration set were identified using a sample selection algorithm in WinISI. The sample selection process identified 105 unique samples based on their position in multivariate space.

5.3. Platform performance

This section describes the methods and techniques associated with monitoring instrument and calibration model stability.

5.3.1. Standardisation

FOSS DA1650 instruments are factory standardised. Systems are maintained against this standard with the use of an external reference correction (ERC) and external wavelength correction (EWC). This was demonstrated with a validation plot of a transfer calibration.

5.3.2. Maintenance requirements – hardware

Hardware maintenance for the DA1650 instruments is minimal, but diagnostic and photometric tests provide confirmation of instrument response with time. They are a combination of processes provided by the instrument manufacturers and SRA NIR spectroscopy specialists. This section describes how each of the tests are conducted.

5.3.2.1. Instrument diagnostic test

The instrument diagnostic test is automatically run on start-up of the instrument. It can also be run manually via the settings menu on the DA1650 instrument.

5.3.2.2. Instrument stability

Each instrument comes with a unique check cell. For each instrument, this cell was analysed against the appropriate prediction model routinely throughout the project life.

A set of six sugar-impregnated fluorilon standards were analysed on the SRA and Tully Mill DA1650s throughout the duration of the project. The standards were analysed by placing them in a small cup and collecting the spectra. Each standard was removed from the cup and replaced, then re-scanned several times.

The spectral data was extracted from Mosaic. Chemometric analyses such as spectral pre-treatments, PCA and PLSR were conducted in The Unscrambler® Software version 10.4 X and WinISI 4.0.

5.3.2.3. Linearity and photometric tests

A set of eight photometric standards were scanned on the Tully Mill and SRA instruments several times over the course of the project. They were analysed in the same manner as the fluorilon sugar standards described in Section 5.3.2.2.

The 10 %, 5 % and 2 % standards were analysed using the *Molasses* data collection method. Due to their high absorbance, a longer integration time was required than is captured in the standard instrument set-up.

5.3.2.4. Instrument calibration (EWC and ERC)

Several discussions were held with spectroscopy and NIR spectrometer hardware specialists at FOSS in Australia and Denmark. The procedure for conducting an instrument calibration for a DA1650 is captured in The FOSS DA1650 User Manual.

5.3.2.5. Known hardware issues

Not applicable.

5.3.3. Maintenance requirements – calibration models

Not applicable.

6. RESULTS AND DISCUSSION

Section 6 contains the results for the experiments described in Section 5. And provides a discussion of the results in relation to current literature and industry factors.

6.1. Transfer and development of standard NIR spectroscopic calibration models

This section describes the development of standard NIR spectroscopic models for the Australian sugar industry. First, transfer models were developed, which are models that contain data collected on one or more platforms, but installed on a different instrument or instrument type. They are typically temporary until additional data from the new instrument can be included in the model. Second, global models refer to calibration models that contain data from multiple instruments, instrument types or mills. Typically, these have slightly higher error than local calibrations, but provide robustness and improve the standardisation of the network. Third, local calibrations are models that comprise calibration data from a single instrument collected at a single mill. These typically provide the lowest errors, but are specific to the mill for which they were developed.

6.1.1. Large-scale preliminary sampling

The development of the calibration models for the FOSS DA1650 was conducted in conjunction with standard mill operations. Generating the data for robust models to be developed, without affecting the standard operation of the mill laboratories required leniency with sampling best practice for this preliminary sampling. Sampling capitalised on the mills' standard daily activities by collecting sample residues from routine testing in the 2014 season. Samples and the associated reference data were sent to a central research facility for NIR analysis. Data was also sourced from the FOSS IX in Mill 5 and DA1650 in Tully Mill. Consequently, the data used to generate the global calibrations was highly variable and included multiple instrument platforms, mills, reference methods and locations.

During the 2014 crushing season, all milling groups were contacted and offered the opportunity to participate in the project by collecting and sending samples to the laboratory in Brisbane for analysis. The response was incredibly strong and 12 mills from six milling groups agreed to participate, which totalled approximately 40 % of the cane supply, based on 2013 numbers.

They included:

- Mossman Mill, Mackay Sugar
- Mulgrave Mill, MSF
- South Johnstone Mill, MSF
- Tully Mill, COFCO
- Inkerman Mill, Wilmar
- Plane Creek Mill, Wilmar
- Millaquin Mill, Bundaberg Sugar
- Condong Mill, NSW Sugar
- Broadwater Mill, NSW Sugar
- Harwood Mill, NSW Sugar
- Isis Mill, Isis Central Mill

= 12,242,502 tonnes cane (2013)

Most mills provided several shipments of a majority of the key products: prepared cane (cane product following the shredder but prior to number one mill processing), bagasse (mills 1 - 5), juice, syrup, molasses (A, B and F), massecuite (A, B and C), magma and raw sugar. While some provided only a selection of products, specific to their needs. Mud samples were provided by only a selection of mills as they're a low-priority product; however, most provided samples that were too small to be analysed by the NIR instrumentation. In total, approximately 2,550 samples were received, which exceeded expectations.

There was a risk that the juice samples were not suitable for calibration purposes due to bacterial activity during transport. The low moisture content of most of the mill products meant they didn't freeze well or hold their cool during transport to the Brisbane laboratory. Additionally, several 'overnight' shipments took over 48 hours to arrive. Subsequent analysis of the spectral data did not show significant variation in the quality of these samples. Outliers were removed according to standard practice.

Despite some issues with sample transport, this procedure allowed a large number of diverse samples to be collected and analysed by NIR spectroscopic and reference chemistry techniques within a short timeframe and provided a good basis for the development of benchtop NIR spectroscopic models for DA instruments.

6.1.2. Development of transfer calibration models

Across the two instrument platforms, 32 transfer calibration models were developed and validated. A summary of the calibration statistics are provided in Table 6 and the validation statistics provided in

Table 7.

Table 6: Summary calibration statistics for transfer calibration models

	Constit.	Instr.	Mean	SD	Range	N	R^2	SEC
Bagasse	Pol	DA1650	3.88	2.51	0.88 - 10.35	811	0.90	0.79
		DA7250	3.88	2.51	0.88 - 10.35	811	0.87	0.90
	Moisture	DA1650	49.45	2.56	43.10 - 55.20	1603	0.80	1.34
		DA7250	49.45	2.56	43.10 - 55.20	1603	0.81	1.30
Juice	Brix	DA1650	11.9	8.11	0.10 – 72.33	942	0.99	0.67
		DA7250	11.9	8.11	0.10 – 72.33	931	0.99	0.55
	Pol	DA1650	31.95	26.93	2.04 – 94.82	1380	1.00	1.84
		DA7250	31.95	26.93	2.04 – 94.82	1380	1.00	1.59
Syrup	Brix	DA1650	70.02	1.72	65.22 – 74.20	386	0.70	0.93
		DA7250	70.02	1.72	65.22 – 74.20	380	0.70	0.93
	Pol	DA1650	63.80	1.81	58.94 – 73.40	385	0.73	0.86
		DA7250	63.80	1.81	58.94 – 73.40	388	0.69	0.92
Molasses	Sucrose	DA1650	42.48	9.17	30.26 – 61.61	623	0.97	1.53
		DA7250	42.48	9.17	30.26 – 61.61	623	0.97	1.53
	DS	DA1650	76.30	1.30	69.60 – 79.00	595	0.61	0.77
		DA7250	76.30	1.30	69.60 – 79.00	589	0.84	0.47
	Brix	DA1650	82.04	5.19	59.06 – 96.92	321	0.80	2.34
		DA7250	82.04	5.19	59.06 – 96.92	321	0.84	2.05
	Pol	DA1650	46.27	8.71	28.54 – 59.10	287	0.97	1.48
		DA7250	46.27	8.71	28.54 – 59.10	287	0.95	1.98
Massecuite	Sucrose	DA1650	68.56	11.96	34.20 – 92.34	826	0.98	1.47
		DA7250	68.56	11.96	34.20 – 92.34	821	0.98	1.57
	DS	DA1650	83.04	3.89	72.04 – 98.17	779	0.92	1.03
		DA7250	83.04	3.89	72.04 – 98.17	783	0.92	1.00
	Brix	DA1650	90.57	9.56	49.6 – 104.6	130	0.94	1.80
		DA7250	90.57	9.56	49.6 – 104.6	130	0.93	2.00
	Pol	DA1650	48.72	13.30	26.92 – 67.49	127	0.94	3.30
		DA7250	48.72	13.30	26.92 – 67.49	127	0.90	4.27
Raw sugar	Pol	DA1650	99.10	0.42	95.10 – 99.8	2089	0.87	0.15
		DA7250	99.10	0.42	95.10 – 99.8	2089	0.91	0.13
	Moisture	DA1650	0.26	0.14	0.06 – 2.51	2091	0.93	0.04
		DA7250	0.26	0.14	0.06 – 2.51	2091	0.93	0.04
Mud	Pol	DA1650	3.10	2.08	0.22 – 21.34	838	0.87	0.71
		DA7250	3.10	2.08	0.22 – 21.34	842	0.84	0.77

SD: standard deviation, N: number of samples, R^2: coefficient of determination, SEC: standard error of calibration, DS: dry substance, Constit.: constituent, Instr.: instrument

Table 7: Summary validation statistics for transfer calibration models

Constit.	Instrument	N	Factors	R^2	SEP	Bias	Slope

Bagasse	Pol	DA1650	1240	8	0.90	1.22	-3.81	0.78
		DA7250	54	6	0.13	2.70	-4.84	0.42
	Moisture	DA1650	1229	6	0.68	2.11	-0.55	0.64
		DA7250	54	7	0.51	2.57	-1.25	0.74
Juice	Brix	DA1650	4	3	0.99	0.64	0.23	1.38
		DA7250	4	3	0.80	0.64	-1.71	0.81
	Pol	DA1650	16	3	0.44	40.38	17.6	0.32
		DA7250	16	5	0.78	15.86	-0.24	0.38
Syrup	Brix	DA1650	53	5	0.32	1.54	0.12	0.50
		DA7250	19	3	0.50	0.96	-1.25	0.81
	Pol	DA1650	53	9	0.25	7.69	3.71	0.51
		DA7250	19	7	0.63	29.09	14.14	0.82
Molasses	Sucrose	DA1650	150	11	0.24	8.35	-1.42	1.02
		DA7250	103	11	0.43	12.11	2.54	0.99
	DS	DA1650	151	2	0.10	1.50	-2.30	0.78
		DA7250	104	6	0.52	2.63	1.03	0.58
	Brix	DA1650	60	3	0.85	2.31	0.27	0.73
		DA7250	51	7	0.58	4.89	1.66	0.46
	Pol	DA1650	39	15	0.87	3.78	-0.03	0.83
		DA7250	39	10	0.40	8.46	1.35	0.24
Massecuite	Sucrose	DA1650	139	13	0.84	3.11	-1.52	0.66
		DA7250	92	9	0.69	5.57	1.48	0.89
	DS	DA1650	140	3	0.96	1.39	-1.10	0.80
		DA7250	92	5	0.73	1.85	-3.64	0.73
	Brix	DA1650	25	4	0.76	2.76	0.58	0.97
		DA7250	25	3	0.80	1.92	-1.33	0.087
	Pol	DA1650	25	10	0.99	2.66	-0.90	0.95
		DA7250	25	3	0.91	6.09	-4.71	0.59
Raw sugar	Pol	DA1650	834	6	0.53	1.72	0.85	0.87
		DA7250	84	9	0.29	0.28	0.09	0.02
	Moisture	DA1650	834	10	0.51	0.13	-0.04	1.42
		DA7250	84	11	0.01	0.88	0.43	0.11
Mud	Pol	DA1650	6	10	0.91	0.64	0.18	1.24
		DA7250	6	6	0.58	1.06	-0.45	1.15

SD: standard deviation; N: number of samples; R^2: coefficient of determination; SEP: standard error of prediction; DS: dry substance, Constit.: constituent, Instr.: instrument

Overall, the direct transfer calibrations were fair, considering the validation samples were generated on a different style of instrument. None of the calibrations were of high enough quality to be used as is, as was expected. Typically, the validation plots had fairly even distributions with a trend line parallel to that of the calibration plot (Figure 1). This is known as bias and is expected when an instrument or mill is not represented in the calibration set. It typically results from an absorbance shift in the spectral data. This is an excellent outcome for a transfer calibration as it can be used as is with a bias correction (mathematical offset) until enough of the local data has been built into the calibration set to remove the offset.

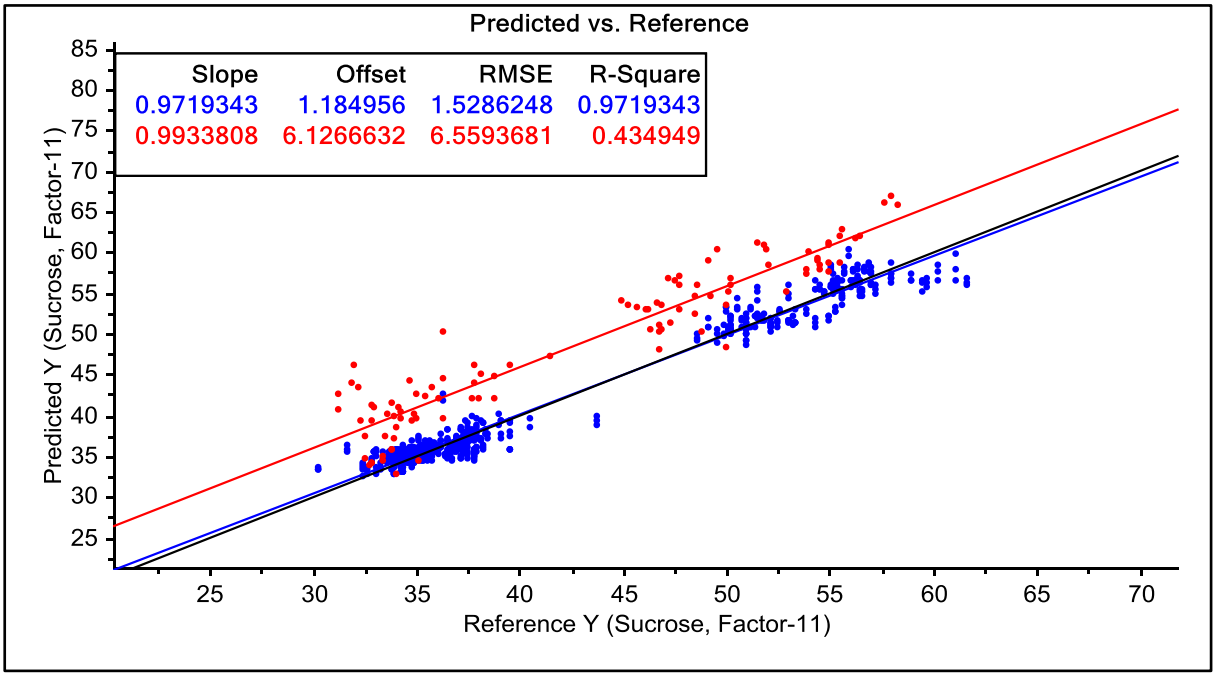

Figure 1: Transfer calibration (blue) and validation (red) plot for sucrose in molasses, IX vs DA7250. Shows parallel bias

At other times, the validation plot overlaid the calibration plot, although the validation plot tended to show slightly more scatter (Figure 2). This often occurred when the range of the calibration set was less than the range of the validation set, and can produce misleading statistics, as illustrated by Figure 3, which shows that the validation samples sit quite tightly on the calibration line, but the limited number of samples skew the validation line. When the validation set sits on the calibration set and there is appropriate range to prevent skew, the model can be used as-is. If skew is present, more samples are required that span the range before the calibration can be trusted.

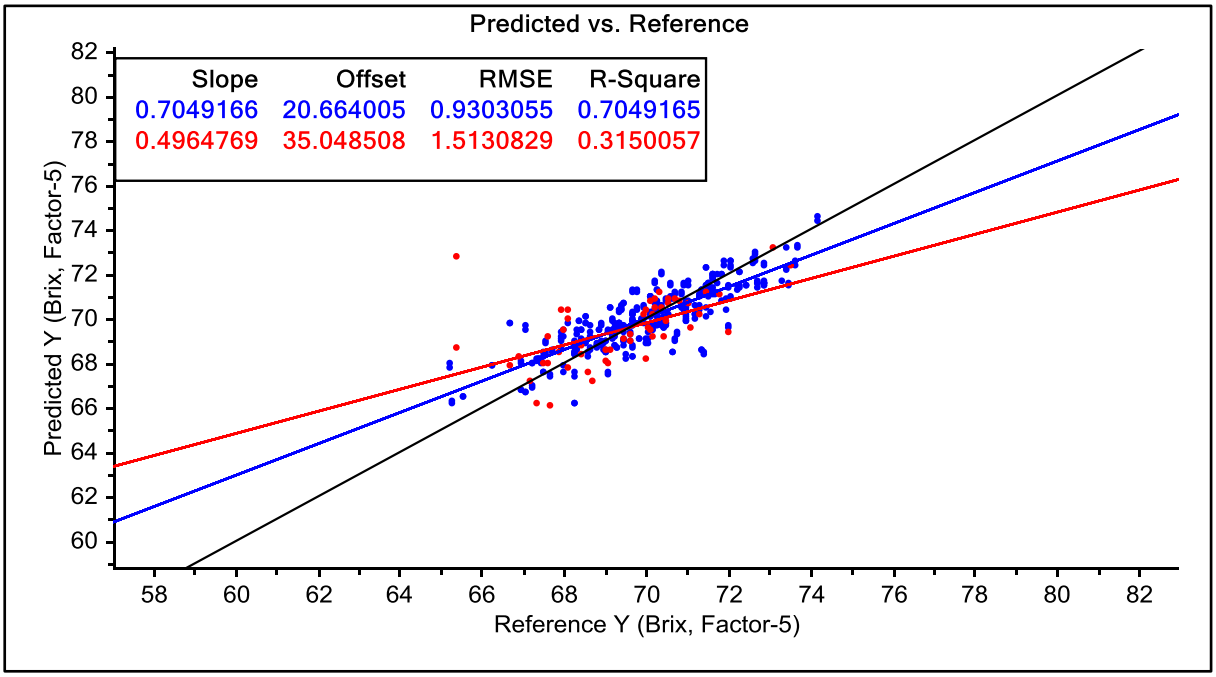

Figure 2: Transfer calibration (blue) and validation (red) plot for brix in syrup. IX v DA1650. Shows good overlay of validation samples on the calibration samples

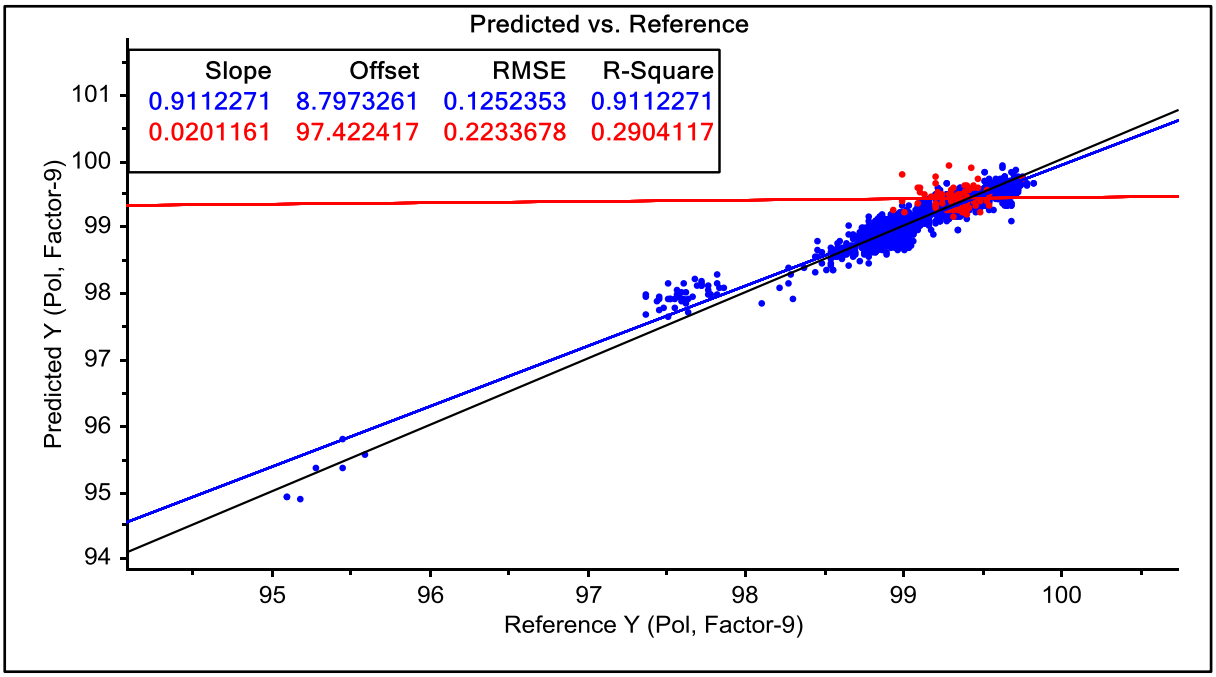

Figure 3: Transfer calibration (blue) and validation (red) for pol in raw sugar. IX v DA7250. Shows skew in validation statistics due to lack of range

Mostly, there was little difference between the prediction errors of the DA1650 and DA7250, although the DA1650 occasionally benefited from having more samples that spanned the range. Neither instrument routinely out-performed the other. Rarely, the validation plots for the two instruments were different, an example of this can be seen by comparing Figure 4 and Figure 1, which are the same calibrations validated with different instruments. This was the worst case of difference. The similarity in the instruments was also illustrated in their treated spectra Figure 5.

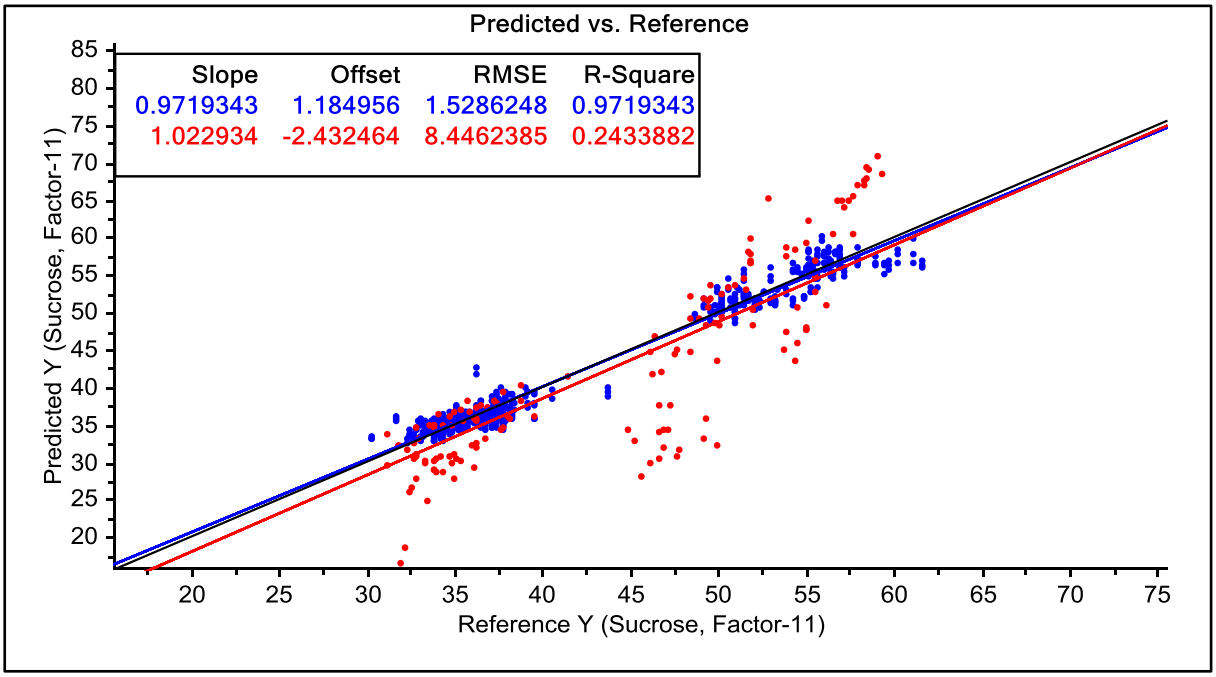

Figure 4: Transfer calibration (blue) and validation (red) plot for sucrose in molasses. IX v DA1650. An example of the difference between the two instruments' predictions, to be compared with Figure 1. Shows no bias, but significant vertical scatter

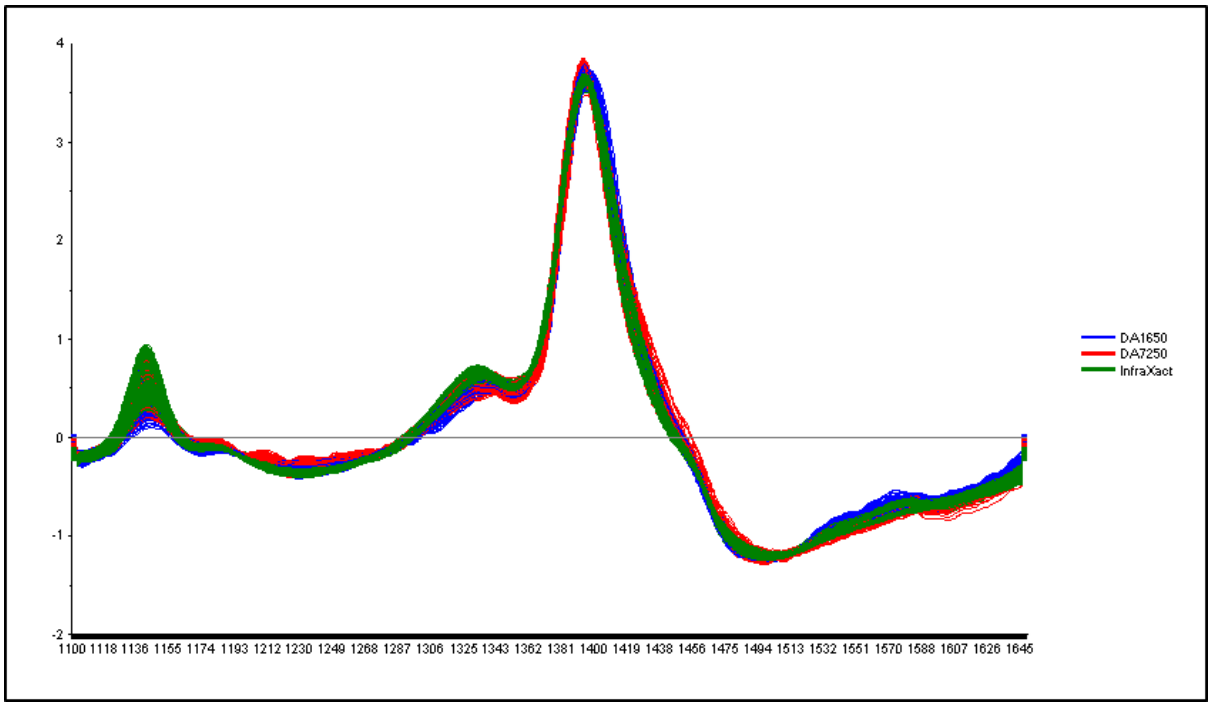

Figure 5: Plot showing the 1st derivative, SNV-treated spectra for bagasse, with the three different instrument sources identified (blue – DA1650, red – DA7250, green – IX). Illustrates the similarity of spectra between instruments

This indicates that both the FOSS and Perten diode array instruments can handle transfer data from a different instrument equally well, but each parameter must be assessed individually. The data shows that transfer calibrations are not always fully effective immediately, and the validation set doesn't respond the same way each time.

It is rare that a calibration would be left in this state for long, however. Normally, data collected on the instrument/ at the mill in question would be included in the dataset to start localising the model immediately following installation.

6.1.3. Development of standard calibration models

The early stages of developing an NIR spectroscopic system requires many iterations in the calibration development phase. As models are developed and validated, this additional data is captured as part of a subsequent calibration set, and validated again. This process repeats until the expansion of the calibration is no longer providing a significant reduction in error and the system is designated fit-for-purpose by the end-user. This section describes the development and iteration of the Global calibration models from 2015 to the current 2017 models.

6.1.3.1. Global 15.0

The Global 15.0 models were developed in Unscrambler®, as an indicator of model performance on the DA1650 and DA7250 instruments. The Global 15.0 models were used as a desktop study only. The calibration and validation statistics are provided in Table 8.

Table 8: Calibration and validation statistics for Global 15.0 calibration with independent validation

Product	Constituent	Calibration			Validation				
		N	SEC	R^2	N	SEP	R^2	Bias	Slope
Bagasse	Pol	2018	0.92	0.92	86	1.06	0.67	-1.2	0.82
	Moisture	2799	1.48	0.80	86	2.56	0.77	0.65	0.59
Juice	Brix	949	0.61	0.99	8	1.21	0.88	-0.81	1.15
	Pol	1412	1.69	1.00	8	80.77	0.63	37.76	4.67
Syrup	Brix	460	1.11	0.62	14	1.29	0.47	-0.53	0.56
	Pol	488	1.04	0.66	14	5.30	0.63	2.53	0.82
	Sucrose	36	0.57	0.93	10	2.10	0.65	0.85	0.43
	DS	36	0.82	0.82	10	0.43	0.79	-0.17	0.89
Molasses	Sucrose	977	2.37	0.93	46	2.79	0.92	-0.20	0.92
	DS	902	0.84	0.79	40	0.93	0.70	-1.92	1.00
	Brix	396	1.71	0.88	30	2.65	0.93	0.96	0.84
	Pol	339	1.80	0.96	30	4.13	0.63	-3.87	0.87
Massecuite	Sucrose	957	1.74	0.97	104	2.39	0.91	-0.96	1.06
	DS	919	1.02	0.95	104	1.54	0.95	0.43	0.90
	Brix	121	0.91	0.98	50	4.39	0.35	0.76	0.16
	Pol	122	1.84	0.98	50	5.58	0.95	-1.17	0.67
Raw sugar	Moisture	2918	0.04	0.83	76	0.05	0.81	0.01	0.27
	Pol	2923	0.15	0.85	76	0.45	0.13	0.20	0.43
	Ash	590	0.01	0.62	6	0.05	0.80	0.02	0.04
	Colour	611	78.9	0.82	34	151.8	0.25	-309	0.41
	RS	579	0.02	0.76	6	0.02	0.20	-0.14	0.52
Mud	Pol	850	0.83	0.82	12	2.78	0.23	1.08	1.14

N: number of samples; R^2: coefficient of determination; SEC: standard error of calibration; SEP: standard error of prediction; DS: dry substance; RS: reducing sugars

The calibration models improved across the board when the diode array data was included in the calibration set. A useful estimate of the robustness of a calibration is a comparison of the SEC and SEP. A rule of thumb for a mature calibration is that the SEP should be less than 1.2x the SEC.

For a starter calibration, much like these globals, that limit is pushed out so the SEP should be less than twice the SEC. Most of the Global 15.0 models fall into that category. Only nine of the 22 calibrations have an SEP that is greater than twice the SEC and of these, six were pol calibrations, which all showed some bias.

Calibrations for pol were also poorer quality in the transfer equations. Pol is often the first parameter to reflect deterioration in the sample, which usually presents a strong bias in one direction. While we see this with juice in Figure 6, the small size of the validation set makes it unclear whether this is due to deterioration or a real bias related to instrumental or mill differences. Of all of the products, juice would be the most likely to suffer deterioration due to its low (relatively) brix content.

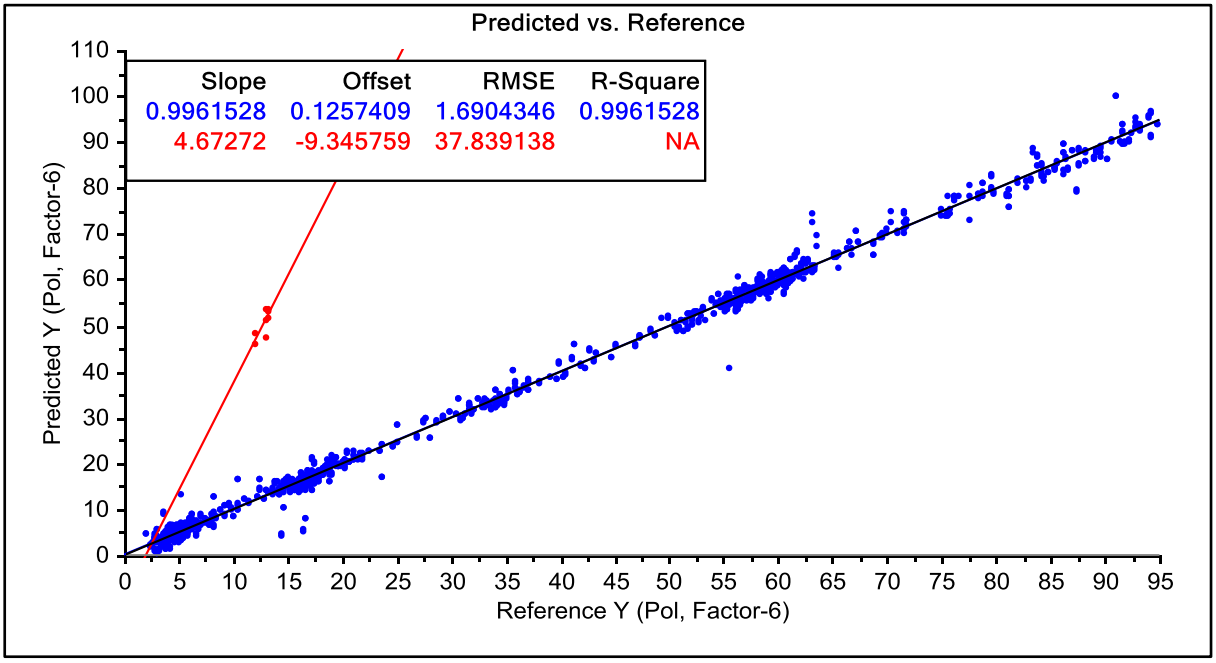

Figure 6: Global calibration and independent validation of pol in juice, showing strong bias

The plot of the massecuite and magma calibration (Figure 7) shows why the SEP (5.58) was so poor. The range of the validation set extends much higher than the calibration set and the range of calibration set extends much lower than the validation set. The typical range in pol for these products is between 35 % and 90 %. The meant that the high-pol samples such as magma, A massecuite and B massecuite had large amounts of scatter about the regression line. This calibration suffered from the poor distribution of the available data, however, that the linearity was maintained when predicting high-pol samples indicates the model is likely to improve with improved representation over an extended range for the calibration population.

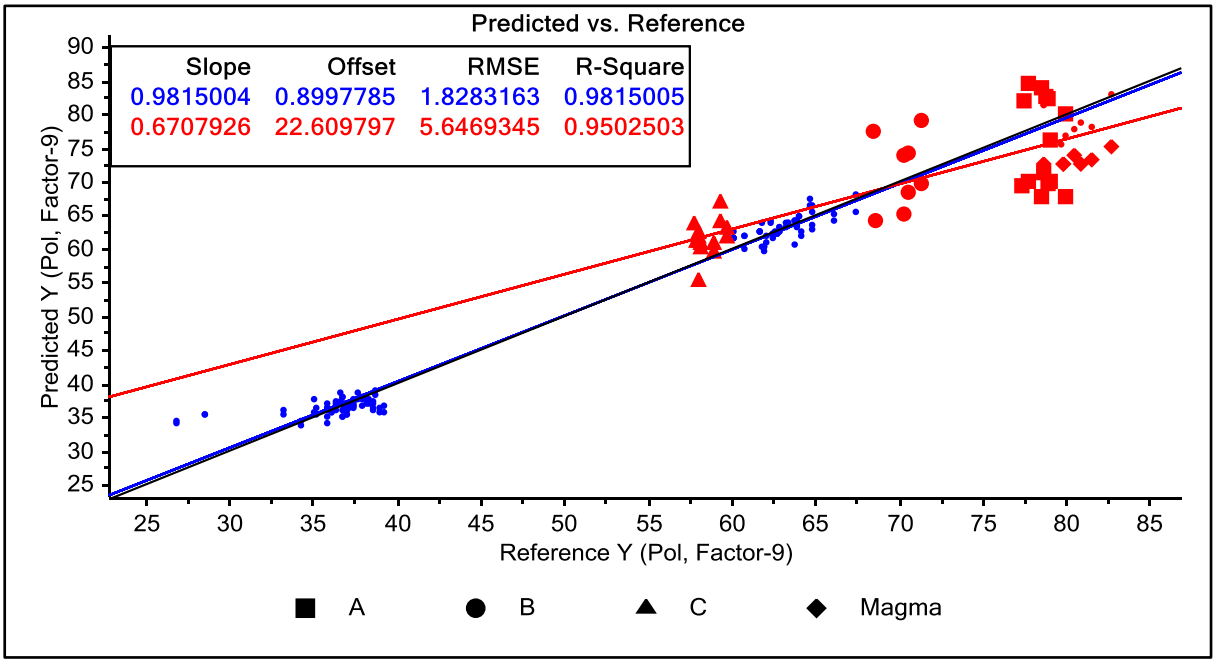

Figure 7: Massecuite and magma calibration showing the different products.

The remaining pol calibrations showed typical causes for high SEP values; mostly bias as seen in Figure 8. The bias appears to be exacerbated by the presence of the two instruments in the validation plot. Really, each instrument has their own bias; the circles, mostly above the red regression line represent the DA7250, with a moderate bias, whereas the squares, which represent the DA1650 and are mostly below the red regression line, on their own would show very little bias.

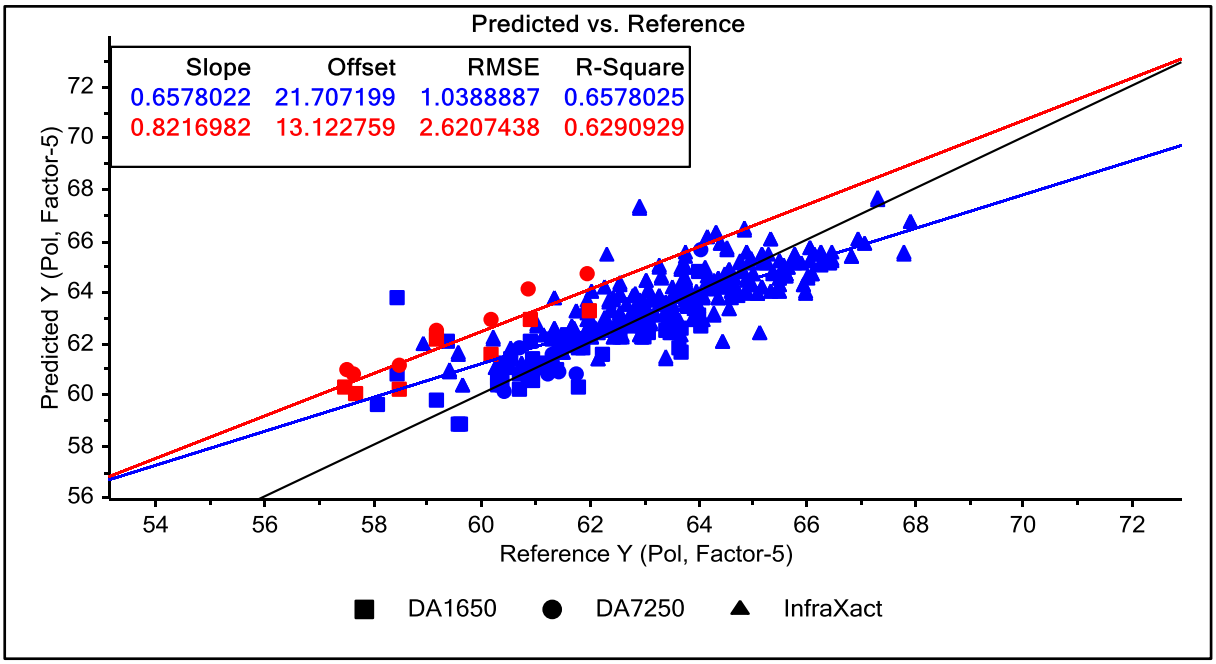

Figure 8: Global calibration and validation for pol in syrup showing bias.

Alternatively, most of the remaining calibrations performed very well as illustrated by the calibration and validation plots, which overlaid nicely and the statistics, which were within the expected limits. Brix in juice in Figure 9 and sucrose in molasses in Figure 10 are representative of these. Overall, the Global 15.0 calibrations showed very promising results and will provide valuable bridging calibrations until local models can be developed. The global models will provide each mill undertaking a trial, predictions that are close enough to get a feel for the quality of the instrument, and identify value while the calibrations are localised. During the trials, we will work with the mills to identify the tolerance limits for each parameter to ensure the results provided will meet appropriate accuracy and precision targets.

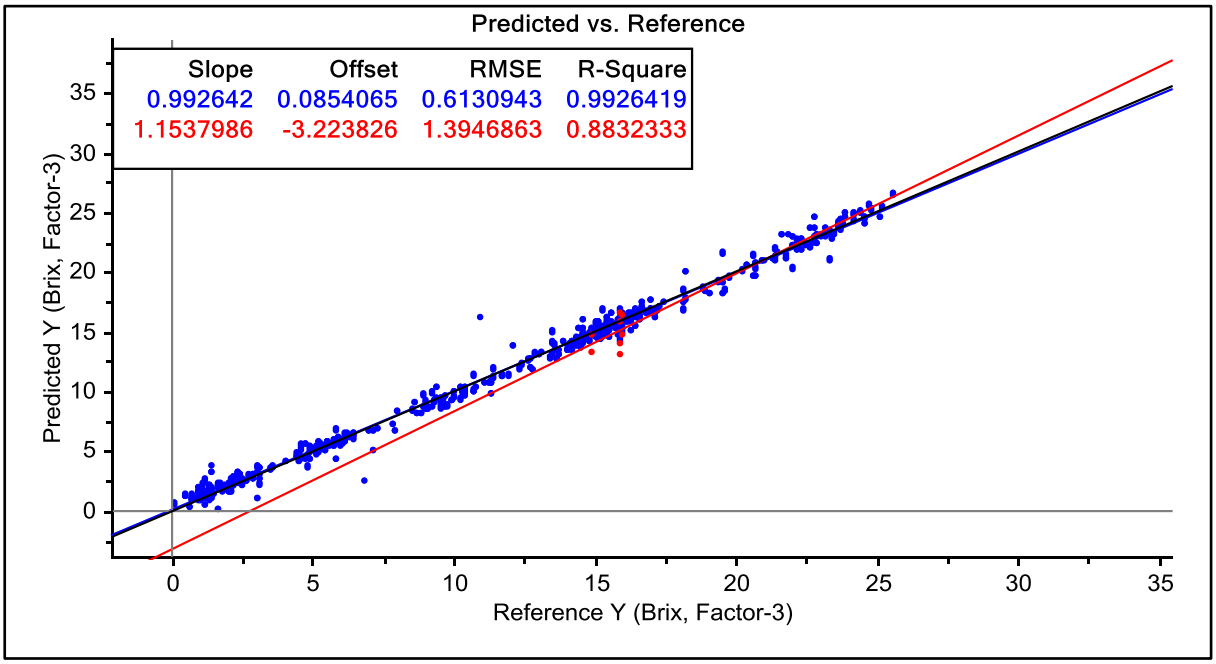

Figure 9: Global calibration for brix in juice

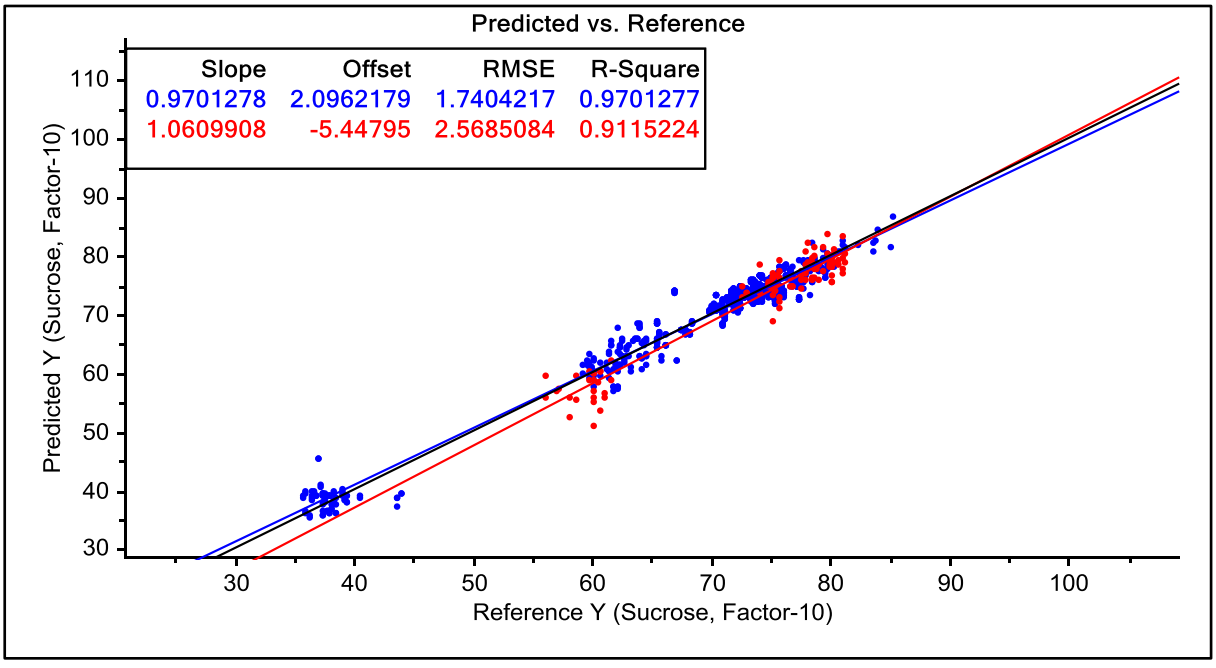

Figure 10: Global calibration for sucrose in molasses

6.1.3.2. Global 15.1

Global 15.1 models were developed and validated for each instrument and the model statistics for the DA1650 and DA7250 are provided in Table 9 and Table 10, respectively. Each system was subsequently trialed in the milling environment.

The bagasse models were divided into primary mills and final mill for pol. The pol values for the final mill range from approximately 0.4 % to 4.5 %, whereas the full milling train ranges from approximately 0.4 % to 13 % and has an SEP of 0.71.

Typically, the DA1650 calibration models outperformed the DA7250 models. Exceptions included molasses pol, raw sugar pol and moisture, and juice brix and pol.

There are several reasons why the DA1650 may have outperformed the DA7250. First is calibration population size. FOSS has been a presence in the Australian sugar industry for much longer than Perten. Unlike the DA7250, the DA1650 has been trialled in multiple mills and environments prior to this project and consequently, more data is available for calibration development. Additionally, the FOSS InfraXact™ instrument has generated large amounts of data over the last 5 - 10 years, albeit a different instrument type.

The InfraXact™ and DA1650 both analyse the sample from below, through a glass-bottomed cup, whereas the DA7250 analyses the sample directly, from above. Each technique has advantages and disadvantages, which will be discussed further later.

The chemometrics used to develop the calibration models is very powerful at removing noise or variation unrelated to chemical changes in the sample. Typically, this means variability in sample presentation and instrument type (e.g. diode array vs monochromators) within a calibration dataset is OK. However, you ideally want the number of additional data to be equal to or less than the number of target data so as not to swamp the features of the instrument being calibrated for (the DA1650 and DA7250, in this case).

When selecting data for the calibration sets, the DA1650 data were supplemented with the InfraXact™ data as the instrument detection was the only variation. Alternatively, the DA7250 calibration set did not include the InfraXact™ data as the sample presentation as well as the instrument detection were different.

Generally, the larger the calibration population size, the more robust the model as more variation is accounted for.

The second factor likely to influence model performance is sample presentation. As mentioned previously, the DA1650 utilises glass-bottomed cups for bottom-up analysis of each sample. This technique provides a flat sampling surface with some degree of compaction in the cup, giving even sample presentation. Small and large cups are available for different products and can be analysed in static or mobile modes. A gold reflector is available for transparent samples to achieve a consistent pathlength of 0.4 mm. However, this sample presentation also brings challenges. As the cells are glass, they are susceptible to scratching, especially during cleaning of viscous, crystalline products such as magma. Additionally, unless the user takes the time to look underneath the cell, voiding or bubbles in the sample may not be noticed.

The top-down analysis of the DA7250 circumvents these issues, however, it presents several other challenges. In particular, the analysis of bagasse and prepared cane in the open-topped large cup is difficult as the product does not flatten well and gets caught in the read head on rotation of the cup.

Also, detector saturation requires a smaller pathlength than the transflectance cell is designed for when analysing juice and syrup samples. The operator is required to manually load the cell with equivalent volumes of sample for each analysis, making it prone to large amounts of error. Overall, the variability in surface due to the open face of the sample cup may be the source of large amounts of error in the model. Despite working with Perten to re-design cups better suited to analysis of sugar factory products, at the conclusion of the project no alternative was found.

The DA1650 model for juice pol shows high errors because there was significant variability in the reference data, which was unknown at the time of model development. Different mills supplied the juice data in different formats, in particular pol reading (°Z) and corrected pol (%). This was rectified in subsequent models.

Table 9: Calibration and validation statistics for DA1650 Global 15.1 models

Product	Constituent	Calibration						Validation					
		Mean	SD	Range	N	R^2	SEC	N	Factor	R^2	SEP	Bias	Slope
Bagasse and prepared cane	Pol - Primary	4.64	3.24	0.42 - 13.05	1981	0.97	0.53	28	16	0.97	0.71	-0.30	0.92
	Pol - Final	2.05	0.41	0.42 - 2.97	995	0.70	0.22	15	16	0.25	0.25	-0.14	0.58
	Moisture	52.76	3.28	41.4 - 63.99	2778	0.90	0.97	28	16	0.54	1.59	0.05	1.00
	Brix	0.86	0.49	0.48 - 1.42	5	0.92	0.96		2				
	Fibre % cane (PC)	14.34	1.59	11.6 - 19.06	81	0.71	0.85	19	9	0.03	1.91	0.29	1.05
	Pol in open cells (PC)	89.28	4.48	80.7 - 101.9	182	0.73	2.32		13				
Molasses	Sucrose	42.98	9.10	30.26 - 64.37	860	0.97	1.47	28	16	0.98	1.23	-1.19	0.98
	Dry substance	76.25	2.17	66.6 - 92.01	844	0.95	0.49	28	16	0.69	0.73	-0.66	0.94
	Brix	82.47	5.16	59.06 - 98.92	358	0.95	1.12	90	16	0.95	1.27	-1.26	0.89
	Pol	46.41	8.93	30.59 - 64.13	291	0.99	0.93	90	16	0.92	2.93	1.83	1.02
	Reducing sugars	10.99	2.65	8.16 - 18.9	55	0.97	0.43	9	8	0.82	0.61	1.43	0.95
	Ash	13.72	1.00	10.8 - 15.34	41	0.95	0.21	9	7	0.65	0.52	0.95	0.38
Raw sugar	Pol	99.05	0.39	95.1 - 99.82	2882	0.93	0.10	60	16	0.35	0.14	0.03	0.59
	Moisture	0.26	0.09	0.06 - 0.98	2882	0.91	0.03	60	16	0.31	0.05	-0.05	0.40
	Ash	0.21	0.02	0.1 - 0.26	573	0.74	0.01	5	16	0.24	0.01	-0.03	0.78
	Colour	1422	157	762 - 1934	585	0.81	68	28	16	0.35	138.00	176.00	0.39
	Reducing sugars	0.22	0.04	0.07 - 0.31	566	0.83	0.02	4	16	0.59	0.01	0.08	1.18
Juice and syrup	Brix	29.96	27.74	0.1 - 74.2	1374	1.00	0.76	43	16	1.00	1.86	-0.63	1.07
	Pol	39.67	27.06	2.04 - 94.82	1831	0.96	5.47	49	16	0.74	15.24	-3.58	0.61
	Ash	1.64	0.53	0.67 - 2.93	26	0.78	0.24		6				
	Sucrose	60.18	7.50	6.94 - 64.03	55	1.00	0.35	9	8	0.35	0.84	-1.68	0.92
	Dry substance	68.19	1.77	63.8 - 71.4	68	0.96	0.35	9	8	0.31	0.74	-0.75	1.55
Massecuite and magma	Brix	92.20	6.89	81.4 - 100.8	139	0.99	0.59	86	11	0.69	3.79	-2.77	0.56
	Pol	53.05	15.24	33.25 - 82.72	139	0.99	1.33	86	11	0.66	3.13	4.99	0.69
	Dry substance	85.38	4.74	72.04 - 98.17	1049	0.98	0.76	100	16	0.75	0.53	1.16	1.16
	Sucrose	71.05	9.75	35.77 - 92.34	1082	0.98	1.30	98	16	0.98	1.40	1.42	1.10
	Crystal content	43.12	6.95	30.0 - 55.6	68	0.89	2.30		8				
Mud	Moisture	74.02	2.57	66.30 - 81.74	310	0.86	0.98	13	16	0.13	4.75	-3.83	-0.96
	Pol	2.93	1.91	0.20 - 8.66	842	0.93	0.52	9	16	0.83	0.64	-1.11	0.86
	Fibre	7.71	1.19	4.13 - 11.29	303	0.75	0.60		16				
	Mud solids	13.46	1.53	8.87 - 18.06	17	0.70	0.82		4				
	Total insoluble solids	21.22	1.57	16.51 - 25.94	16	0.84	0.61		4				

SD: standard deviation; N: number of samples; R^2: coefficient of determination; SEC: standard error of calibration; SEP: standard error of prediction; PC: prepared cane

Table 10: Calibration and validation statistics for DA7250 Global 15.1 models

Product	Constituent	Calibration						Validation					
		Mean	SD	Range	N	R^2	SEC	N	Factor	R^2	SEP	Bias	Slope
Bagasse and prepared cane	Pol - Primary	4.23	3.39	0.42 - 13.05	381	0.95	0.75	20	7	0.92	1.26	0.31	0.86
	Pol - Final	1.86	0.54	0.42 - 3.38	237	0.67	0.31	8	14	0.60	0.56	0.17	0.37
	Moisture	51.44	3.59	41.22 - 60.89	368	0.86	1.31	22	12	0.47	2.08	-0.15	0.48
	Brix	18.86	4.55	1.6 - 24.91	101	0.92	1.26	8	9	0.91	1.81	0.81	1.29
	Fibre % cane (PC)	16.31	3.75	1.21 - 22.08	103	0.90	1.22	8	9	0.86	2.75	1.15	0.90
	Pol in open cells (PC)	65.55	11.96	30.73 - 73.48	84	0.89	0.67	14	4	0.43	1.31	-0.62	0.71
Molasses	Sucrose	61.32	3.07	55.4 - 68.68	112	0.92	0.68	14	12	0.65	1.63	0.58	0.60
	Dry substance	61.22	1.75	57.15 - 64.03	114	0.80	0.79	18	10	0.59	3.71	1.76	1.33
	Brix	68.22	1.77	63.8 - 71.4	137	0.83	0.73	18	5	0.88	1.69	0.77	1.41
	Pol	44.12	9.19	5.54 - 62.49	444	0.90	2.89	92	9	0.78	3.56	-0.31	1.02
	Reducing sugars	76.09	3.00	66.6 - 91.1	450	0.90	0.91	92	9	0.92	1.33	0.25	0.80
	Ash	80.49	6.16	69.23 - 99.7	257	0.97	1.06	38	11	0.93	2.11	0.91	0.90
Raw sugar	Pol	46.07	10.04	29.07 - 58.96	187	0.90	3.16	38	9	0.88	2.81	-1.79	0.78
	Moisture	15.48	1.97	12.83 - 18.90	55	0.81	0.86						
	Ash	77.93	3.56	70.83 - 91.10	385	0.87	1.23	68	8	0.87	2.11	0.87	0.91
	Colour	90.64	5.70	15.3 - 94.44	387	0.81	0.78	68	8	0.62	0.66	-0.24	0.73
	Reducing sugars	84.97	5.77	77.16 - 96.36	80	0.92	1.65	36	8	0.34	7.06	-2.87	0.50
Juice and syrup	Brix	83.33	4.00	75.88 - 88.48	80	0.88	1.40	36	10	0.01	15.87	7.59	0.86
	Pol	98.99	0.30	96.79 - 99.75	949	0.87	0.10	32	9	0.84	0.12	-0.05	0.82
	Ash	0.27	0.09	0.03 - 0.98	961	0.84	0.04	32	9	0.81	0.041	0.01	0.98
	Sucrose	0.20	0.02	0.12 - 0.26	568	0.67	0.01	32	12	0.08	0.03	-0.02	0.60
	Dry substance	0.22	0.03	0.15 - 0.36	564	0.61	0.02	32	10	0.46	0.24	0.12	0.80
Massecuite and magma	Brix	1422	173	47 - 1934	688	0.73	95.70						
	Pol	4.23	3.39	0.42 - 13.05	381	0.95	0.75	20	7	0.92	1.26	0.31	0.86
	Dry substance	1.86	0.54	0.42 - 3.38	237	0.67	0.31	8	14	0.60	0.56	0.17	0.37
	Sucrose	51.44	3.59	41.22 - 60.89	368	0.86	1.31	22	12	0.47	2.08	-0.15	0.48
	Crystal content	18.86	4.55	1.6 - 24.91	101	0.92	1.26	8	9	0.91	1.81	0.81	1.29
Mud	Moisture	16.31	3.75	1.21 - 22.08	103	0.90	1.22	8	9	0.86	2.75	1.15	0.90
	Pol	65.55	11.96	30.73 - 73.48	84	0.89	0.67	14	4	0.43	1.31	-0.62	0.71
	Fibre	61.32	3.07	55.4 - 68.68	112	0.92	0.68	14	12	0.65	1.63	0.58	0.60
	Mud solids	61.22	1.75	57.15 - 64.03	114	0.80	0.79	18	10	0.59	3.71	1.76	1.33
	Total insoluble solids	68.22	1.77	63.8 - 71.4	137	0.83	0.73	18	5	0.88	1.69	0.77	1.41

SD: standard deviation; N: number of samples; R^2: coefficient of determination; SEC: standard error of calibration; SEP: standard error of prediction; PC: prepared cane

The Global 15.1 calibration models on the DA1650 were trialled at Mill 12 and Mill 11, early in the 2016 crushing season. Trials were also conducted at Mill 1.

A limitation of the trials was data generation. Unfortunately, there were not as many samples analysed during each trial as was expected. Two causes for this were staffing availability at the mill and mill lab throughput. Although NIR analysis is fast, mills rarely analysed all of the samples put through the laboratory. To overcome this in the 2016 season, we asked each mill to stockpile samples prior to the trial and remained on site to help process them in the first few days of the trial.

Figure 11 to Figure 17 shows representative calibration and validation plots, along with the relevant statistics resulting from these trials. For some products, there was significant deviation between the NIR predicted and laboratory reference data. This was particularly relevant for the data from Mill 12, which was not represented in the calibration set.

The bagasse pol model showed very strong slope variations for all three mills (Figure 15 to Figure 17), although the response was linear. The cause of this is unknown. Typically, when the slope value (m) does not approach 1, it suggests a poorly represented model, i.e. the sample variation is not well accounted for. However, this model has 1981 samples with a large range so it's unlikely that this is the cause. Recalibration will required to correct these slope issues.

Overall, the results of the early 2015 trials were promising, but it was evident that the models were not suitable for routine use. The models were updated with additional data to Global 15.2, described in Sections 5.1.3.3 and 6.1.3.3.

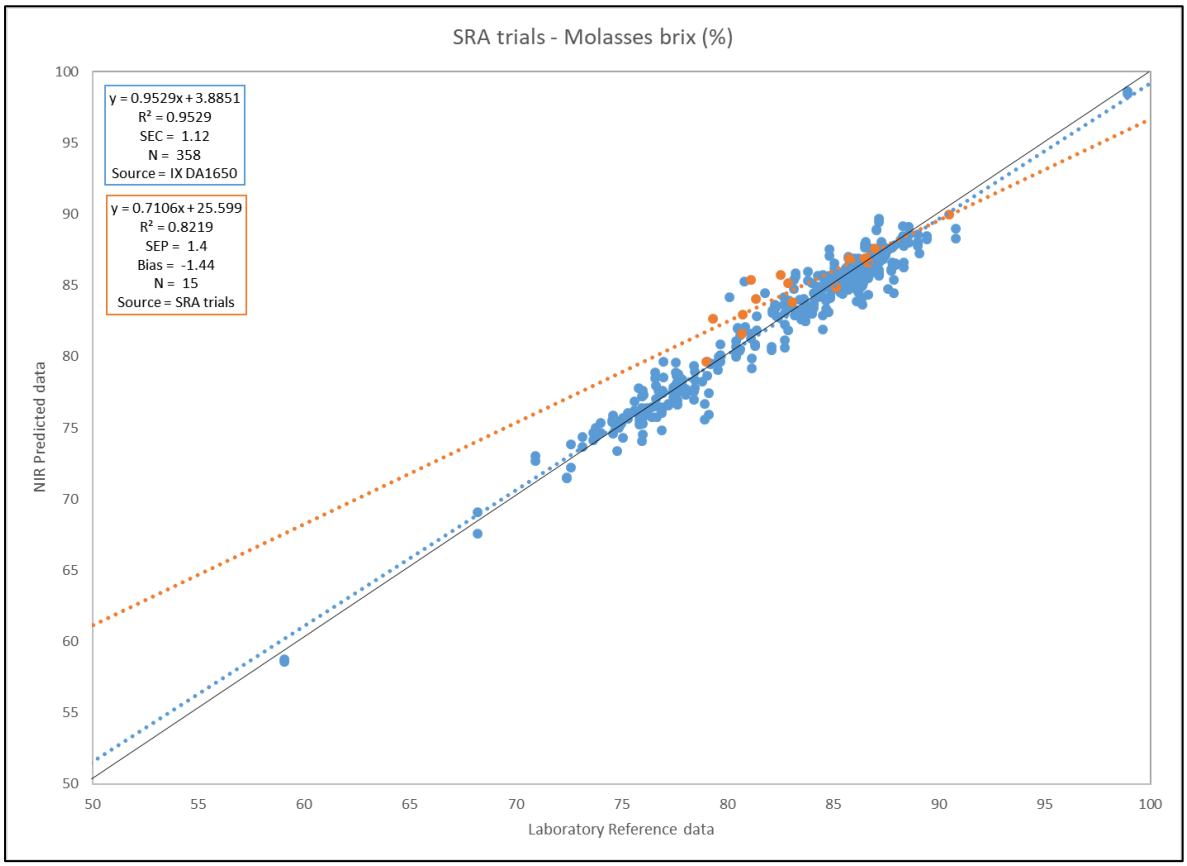

Figure 11: SRA trials' calibration (blue) and validation (orange) plot for molasses brix

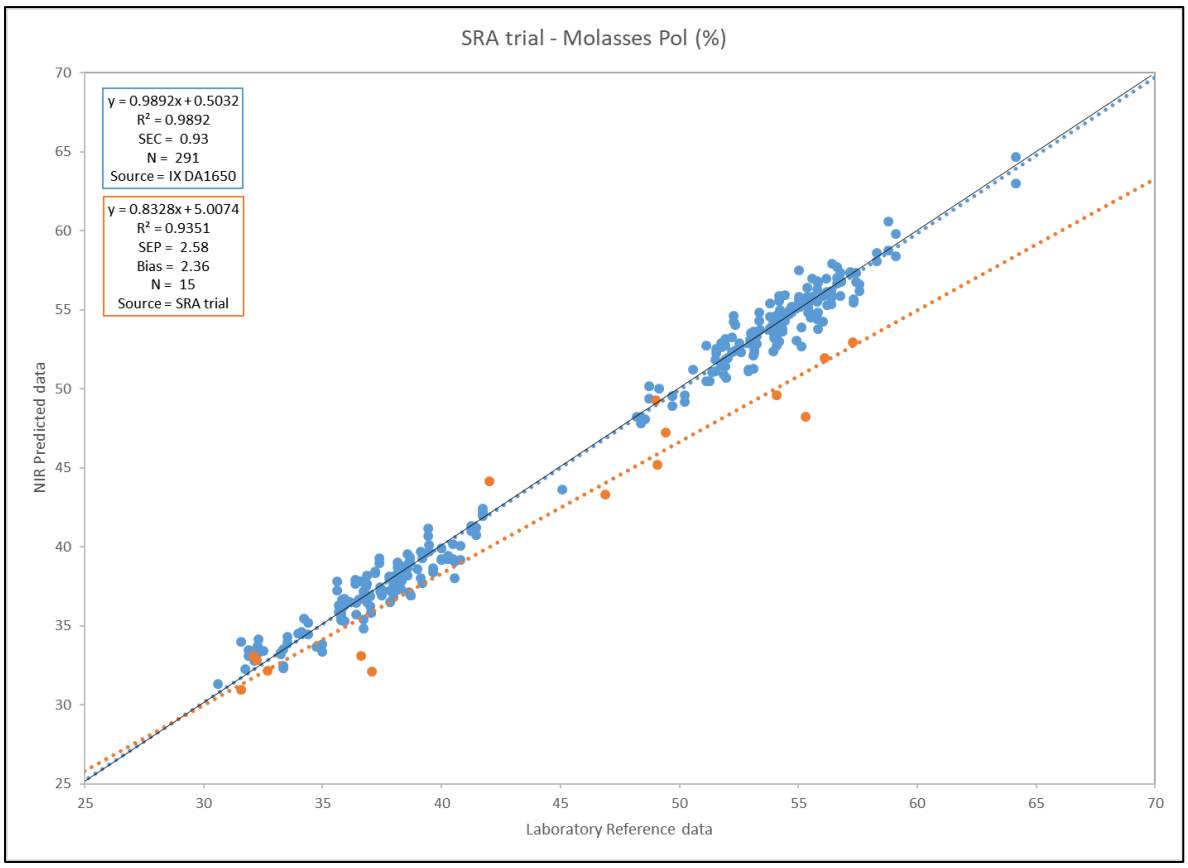

Figure 12: SRA trials' calibration (blue) and validation (orange) plot for molasses pol

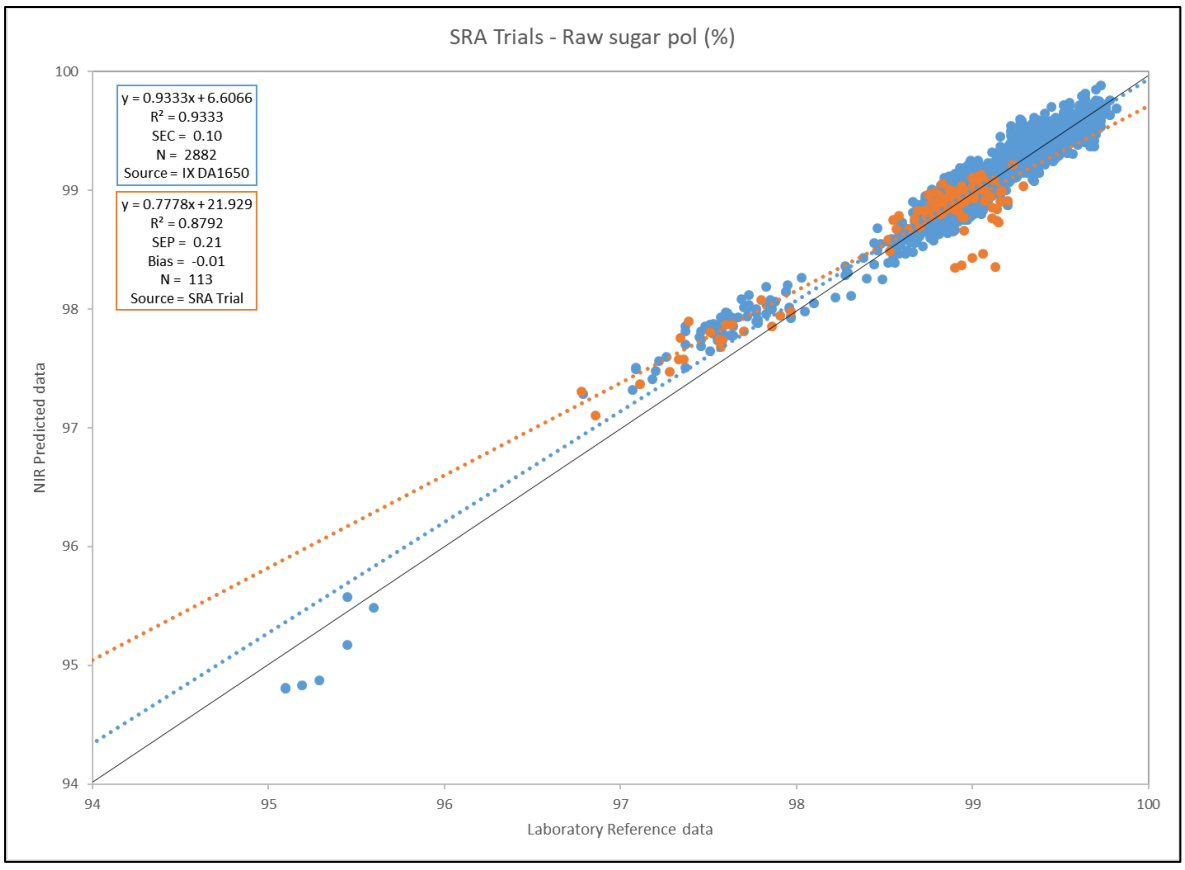

Figure 13: SRA trials' calibration (blue) and validation (orange) plot for raw sugar pol

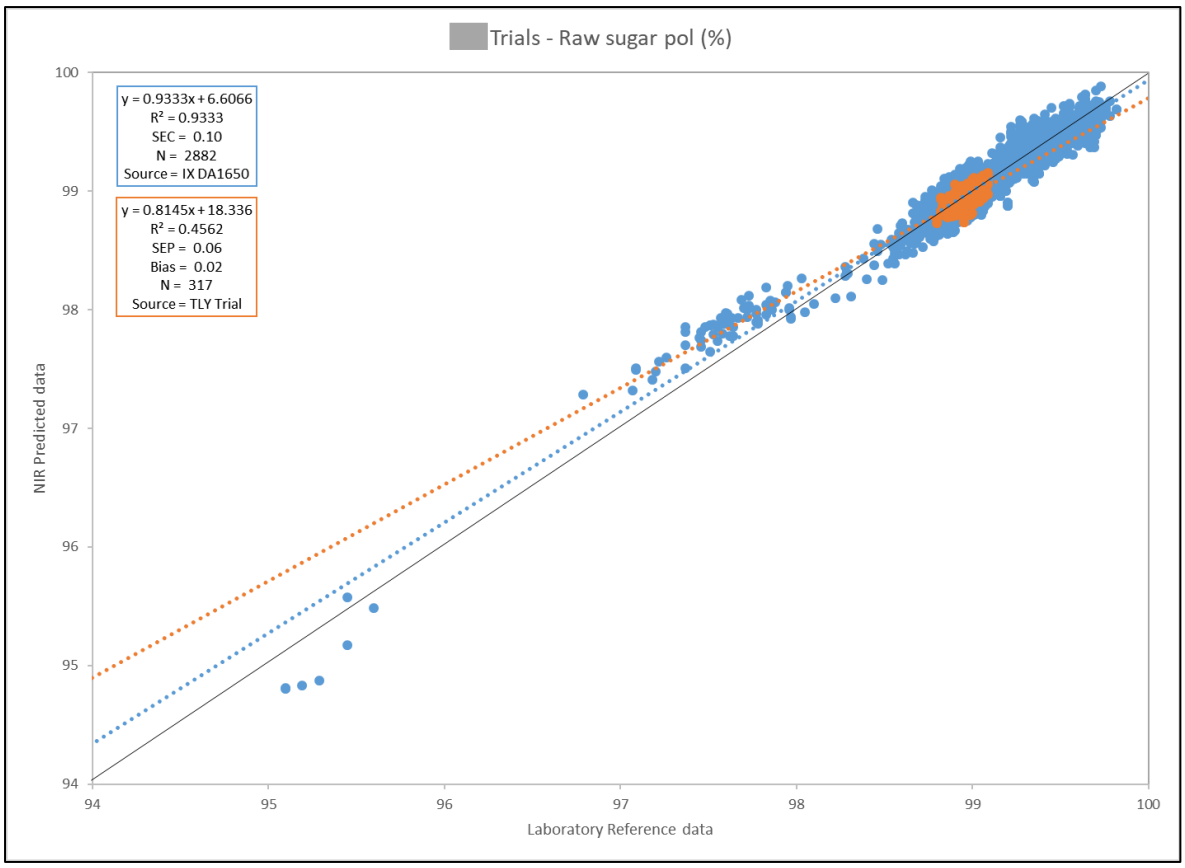

Figure 14: Mill 1 calibration (blue) and validation (orange) plot for raw sugar pol

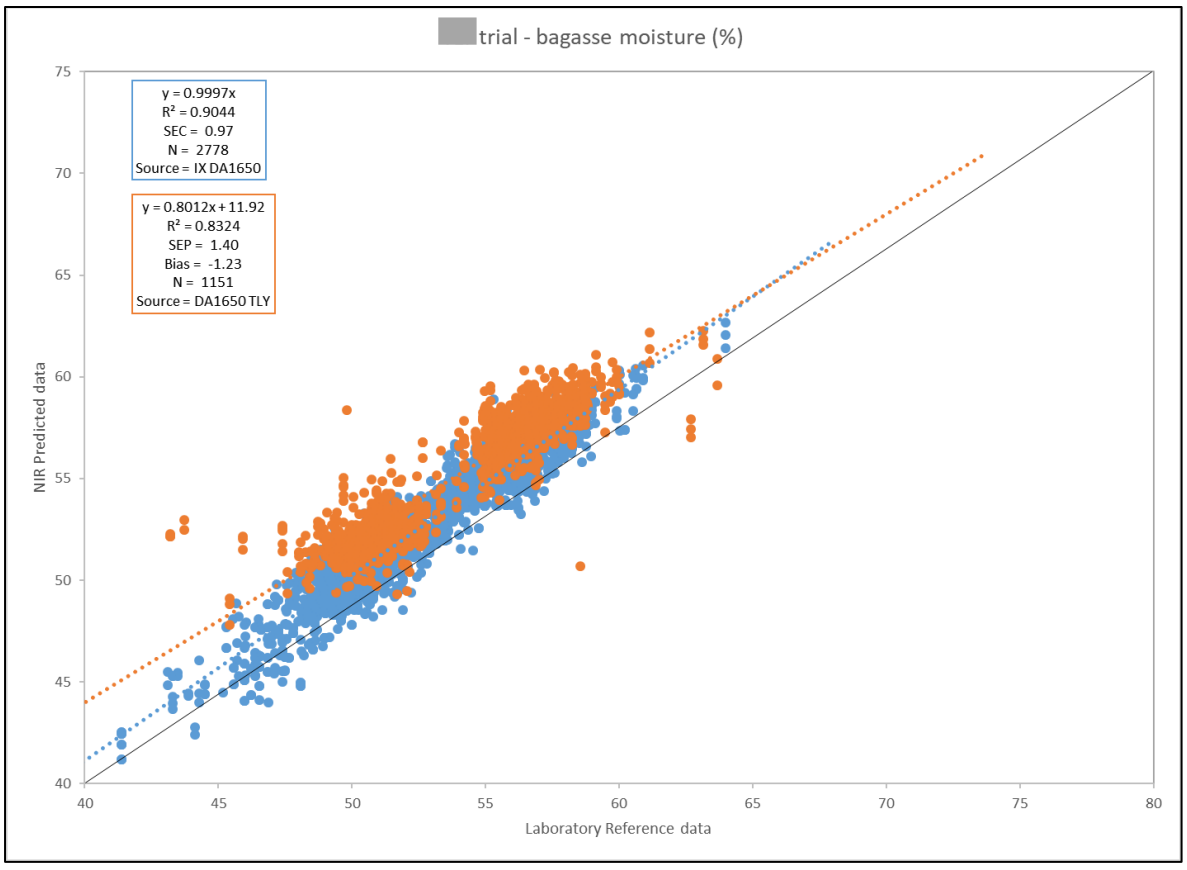

Figure 15: Mill 1 calibration (blue) and validation (orange) plot for bagasse moisture

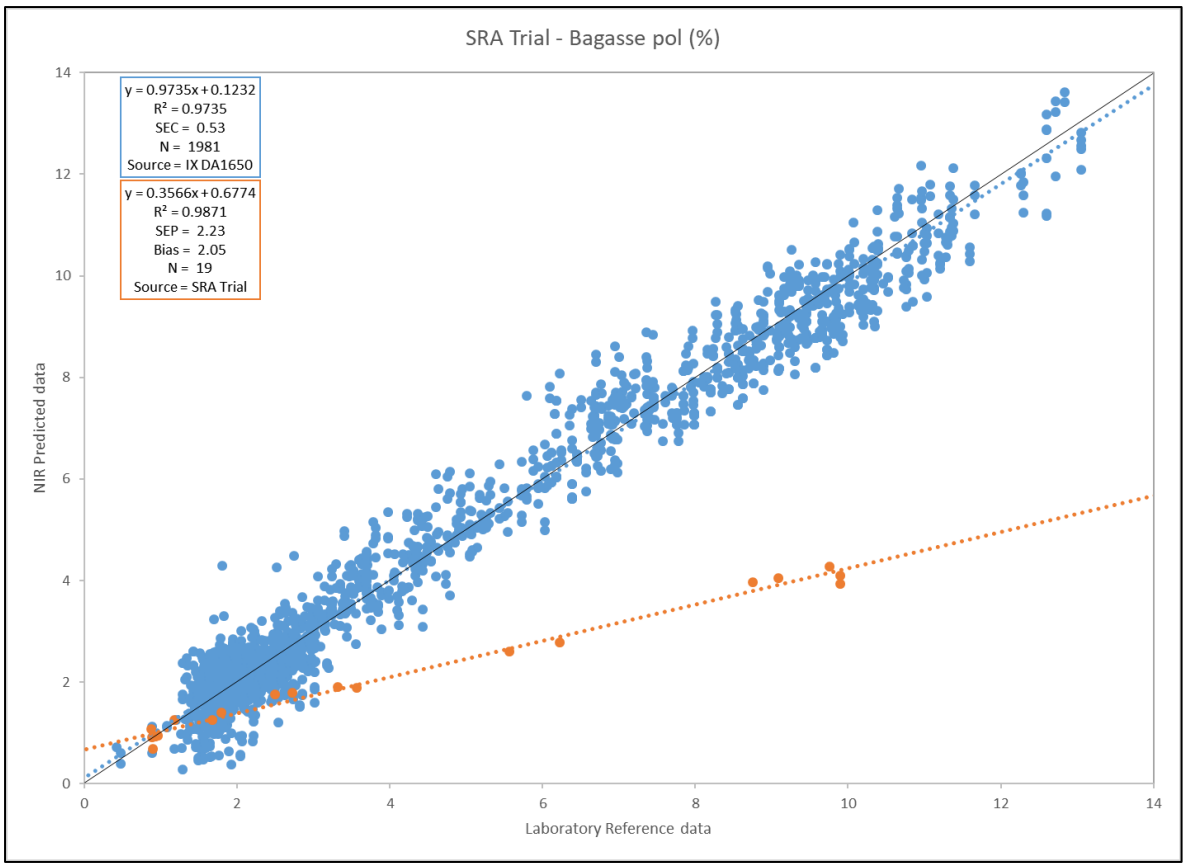

Figure 16: SRA trials' calibration (blue) and validation (orange) plot for bagasse pol

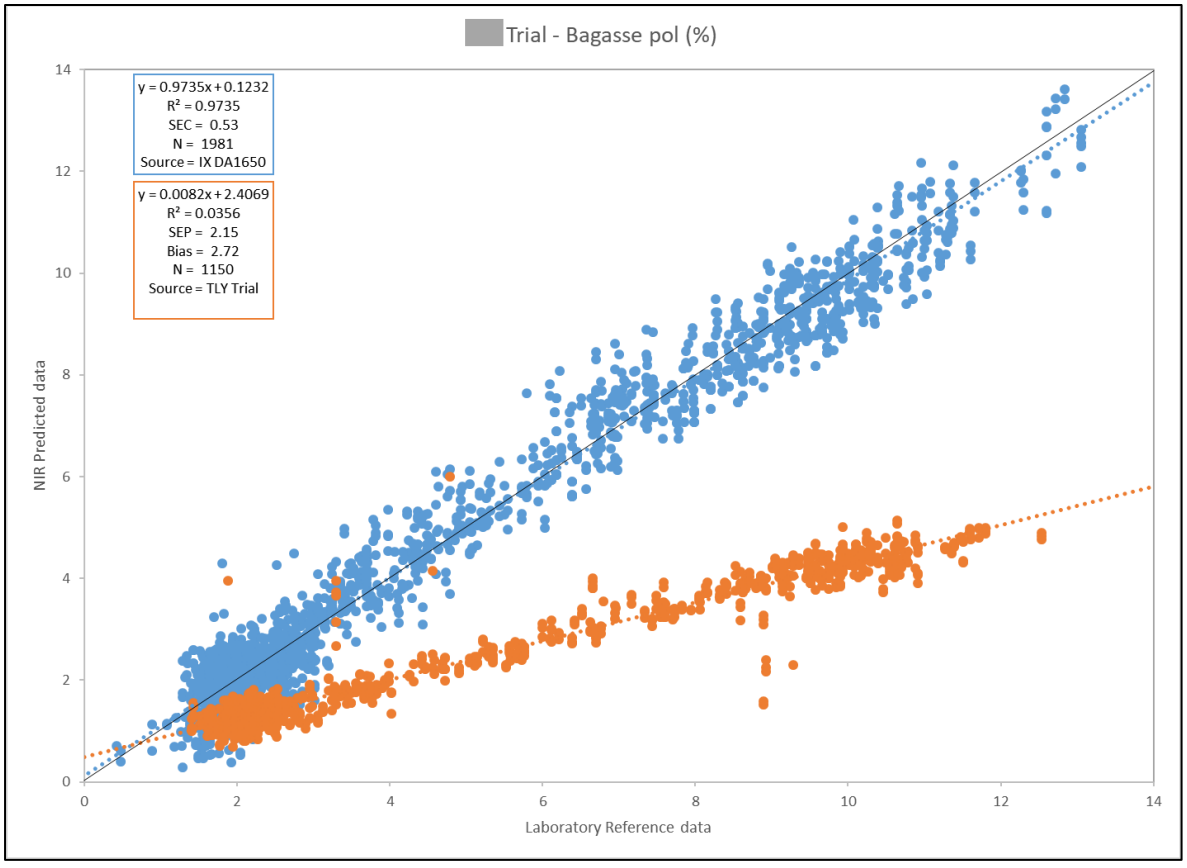

Figure 17: Mill 1 calibration (blue) and validation (orange) plot for bagasse pol

The DA7250 was trialled at Mill 6. Challenges with the instrument sample presentation and software prevented further trials.

The first challenge arose when attempting to load the calibrations on to the instrument. The data matrix of the models did not match the size expected by the instrument. This was due to the removal of the water regions for calibration development and the way Unscrambler® processes this data when developing the models. This was not a problem previously faced by the Perten team, which was surprising as SRA are not the only people to remove regions of a spectrum for calibration.

The models were eventually loaded onto the instrument by deleting the values in these regions but keeping them in the model. Testing within the Unscrambler® software indicated that the predictions under these conditions would be equivalent to those calculated with these regions removed. This was also the solution provided by the Chemometricians at Perten in Sweden.

It became evident immediately after installation at Mill 6, however, that this solution was not an effective one. The predictions for all products' constituents were clearly very wrong; far outside the expected error tolerances of the models, as suggested by the calibration SECs and SEPs. Regardless, Mill 6 continued with the trial to increase their data count. Plots showing the variability in predictive performance are provided in Figure 18 to Figure 24.

The plots show that the instrument predictions are repeatibly incorrect, i.e. they are clustering within the same range. Despite this, linear correlations between the predicted and reference values are not holding; the maximum variability in the predicted data is not due to change in concentration. Rather, there seems to be a distinction for some products that relates to product type. This is particularly evident in Figure 21 to Figure 24, which each show two 'lines' of data in the plot. In Figure 21, the upper line consists of C molasses and the lower line, is A and B molasses. In Figure 23 the upper line consists of A and B massecuite, whereas the lower line is magma.

Considering the calibration development performed in the Unscrambler software was feasible, it is unlikely that the instrument is truly failing to distinguish chemical variability in the samples. It is also unlikely that sample presentation issues in isolation are preventing suitable prediction. Rather, it is proposed that the mathematical treatment of the freshly collected spectra to predict a suitable value is not working as expected. The new spectra are not being treated in the same way as the calibration samples, as the wavelength regions cannot be treated in the same way.

After many discussions with Perten, it was identified that the regions of the spectrum to be removed must be downweighted for each calibration developed. This is a manual process and adds significant development time to each model.

A second trial in 2016 with new sample presentation strategies did not improve the applicability of this instrument to the analysis of sugarcane factory products. Consequently, this instrument has been deemed unsuitable until better presentation can be achieved.

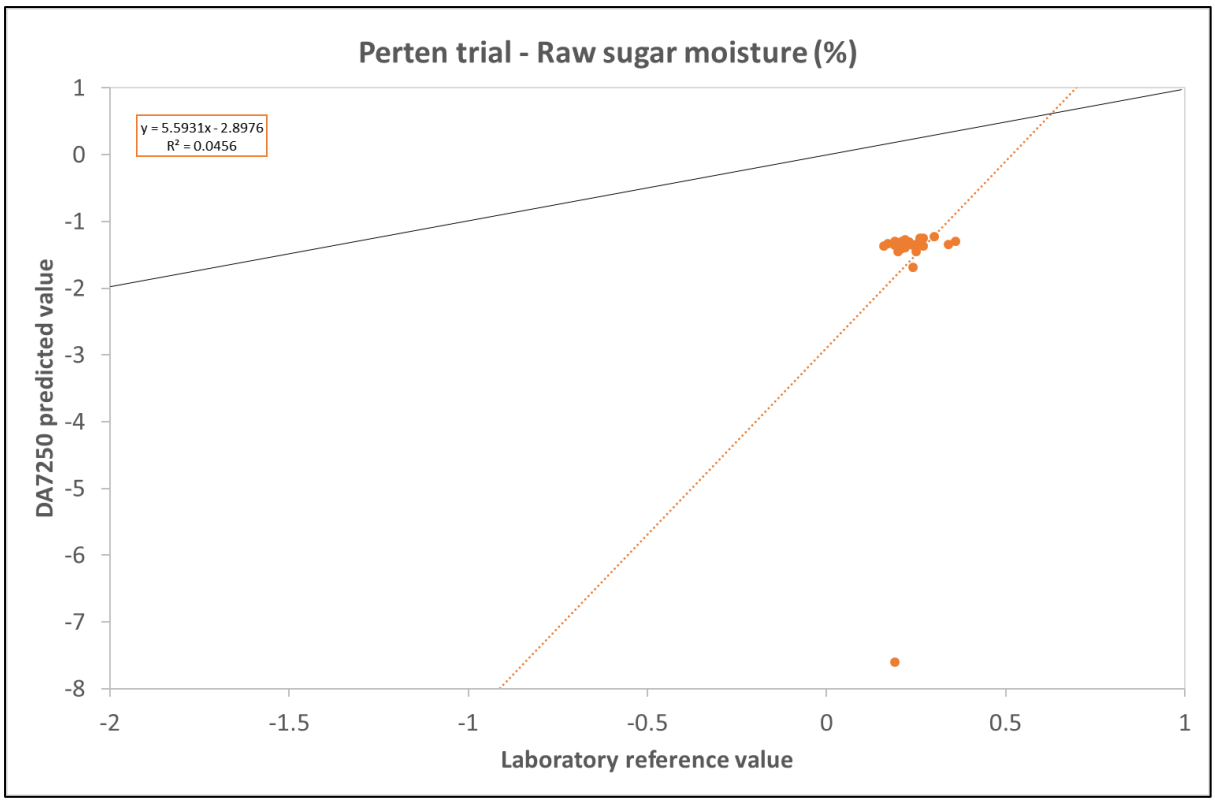

Figure 18: Perten Global 15.1 Mill 6 trial validation – Raw sugar moisture

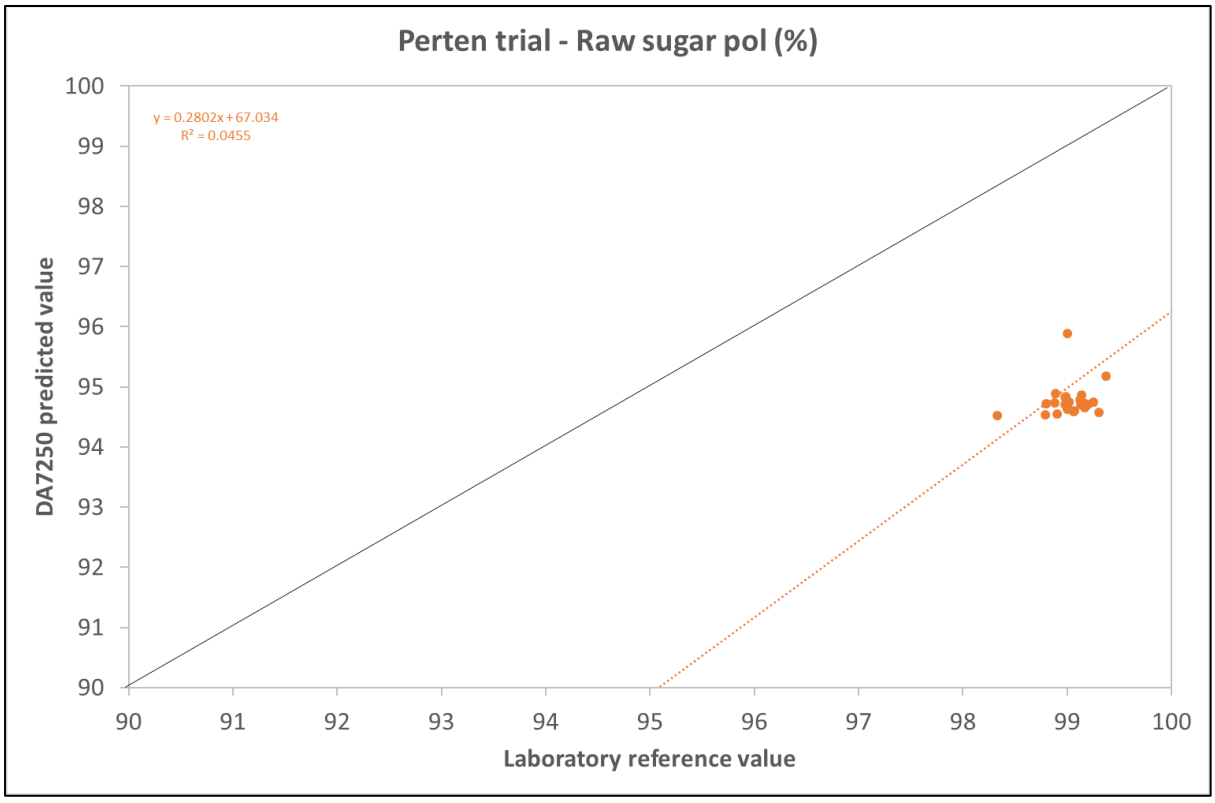

Figure 19: Perten Global 15.1 Mill 6 trial validation – Raw sugar pol

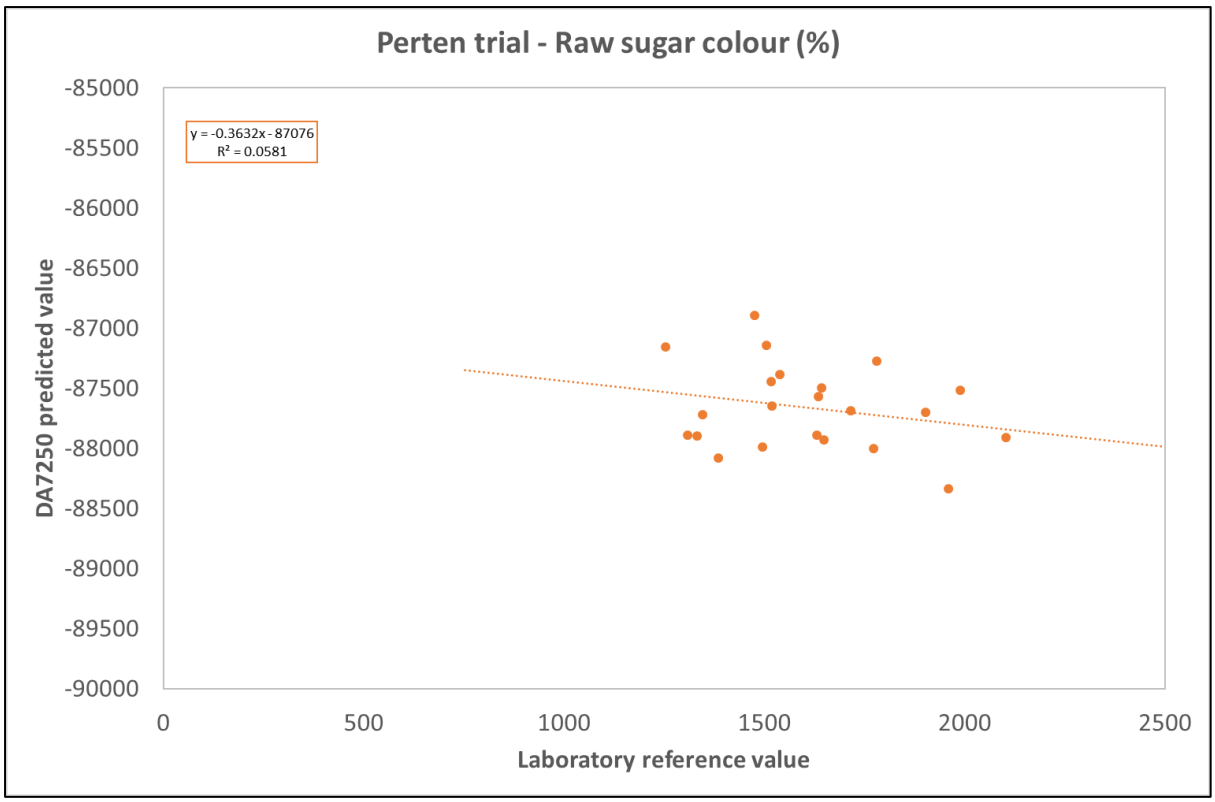

Figure 20: Perten Global 15.1 Mill 6 trial validation – Raw sugar colour

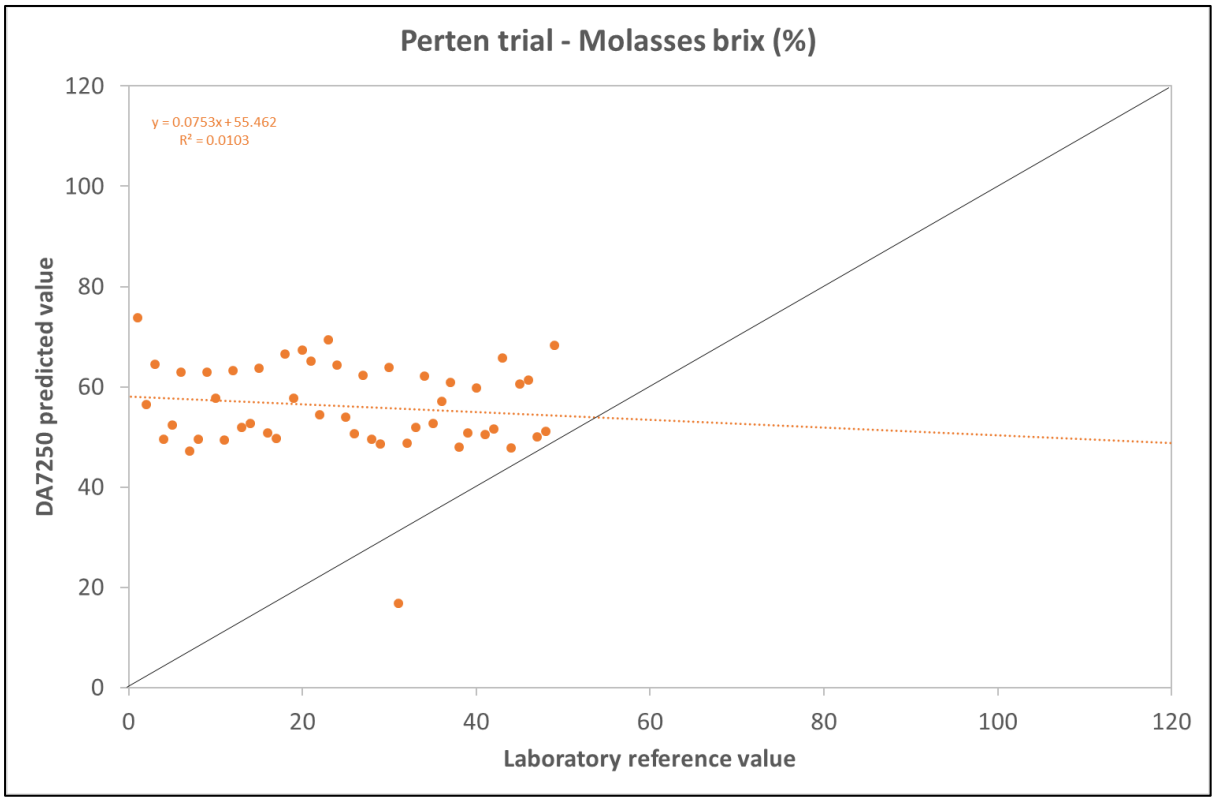

Figure 21: Perten Global 15.1 Mill 6 trial validation – Molasses brix

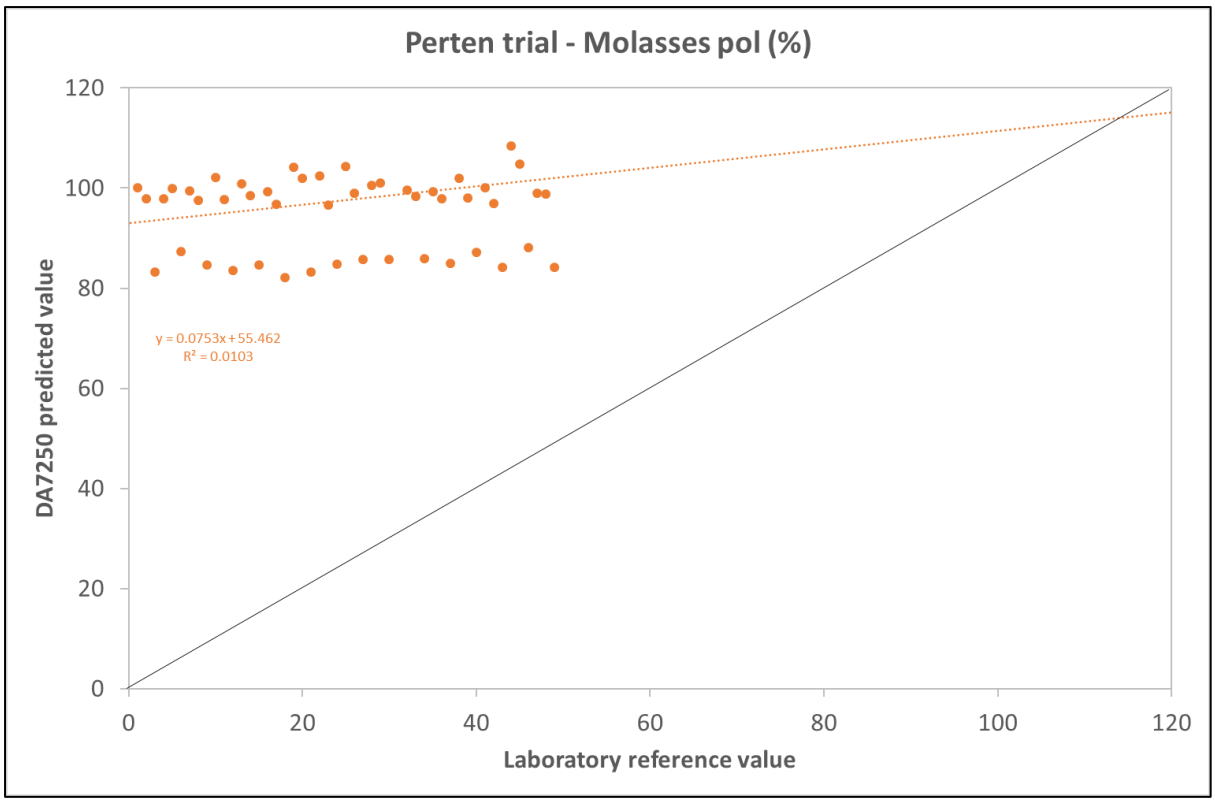

Figure 22: Perten Global 15.1 Mill 6 trial validation – Molasses pol

Figure 23: Perten Global 15.1 Mill 6 trial validation – Massecuite brix

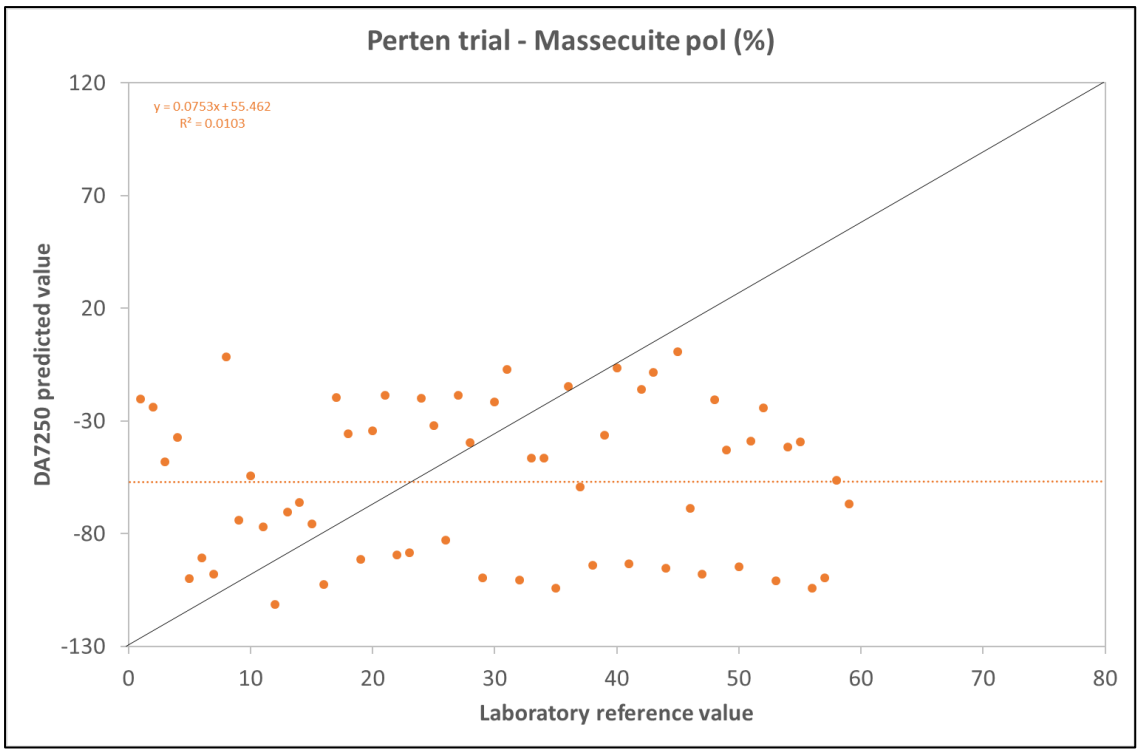

Figure 24: Perten Global 15.1 Mill 6 trial validation – Massecuite pol

6.1.3.3. Global 15.2

The calibration model performance statistics for the Global 15.2 models are provided in Table 11. For nearly all products' constituents, the number of samples in the calibration set doubled, affording large reduction in standard errors of calibration (SEC) and standard errors of prediction (SEP), with appreciable increases in calibration coefficient of determinations (R^2).

These models were trialled in Mill 16, Mill 15 and Mill 1. The validation statistics represent the validation data from all three sites. The SEP values are approaching thresholds that would suggest they are suitable for factory use. Particularly pol and moisture for raw sugar and the sucrose and brix models for molasses.

The associated calibration/ validation plots are provided in Figure 25 to Figure 35. Each of the bagasse models (Figure 25 to Figure 28) show a tight core of data that is well predicted in the validation set, surrounded by a lower frequency of data that has a higher amount of scatter. Additionally, both of the final mill models show a slight amount of skew, with the low values over-predicting. This is reflected in the SEP values in Table 11. The molasses models (Figure 29 and Figure 30) show strong performance for both dry substance and sucrose, although the latter has a slight bias. One outlier is observed in the validation set for dry substance. The validation plots for raw sugar show excellent performance for pol (Figure 31) and moisture (Figure 32); but, increased scatter and slight biases for ash (Figure 33), reducing sugars (Figure 34) and colour (Figure 35). Each of these models have around 2000 samples in the calibration set and the SEP value is close to the SEC value (Table 11). This indicates that the models are mature, but the NIR spectroscopic technique is only moderately successful at measuring these factors. This is most likely a combination of the low analyte concentration and the quality and specificity of the reference methods.

Additionally, colour comprises a suite of chemicals, rather than a specific analyte, which is always difficult to calibrate. Dextran in sugar presents a similar issue. It is possible that expanding the calibration set will improve the predictive performance for colour and dextran.

Table 11: Calibration and validation statistics for DA1650 Global 15.2 models

	Constituent	Calibration						Validation					
		Mean	SD	Range	N	R^2	SEC	N	Factors	R^2	SEP	Bias	Slope
Bagasse	Pol - Primary	4.90	3.21	0.90 - 13.1	3505	0.98	0.41	312	16	0.94	0.65	-0.07	0.92
	Pol - Final	2.10	0.58	0.4 - 4.6	2254	0.87	0.21	290	16	0.13	0.36	-0.18	0.26
	Moisture	52.60	3.40	41.4 - 64.0	5125	0.94	0.85	312	16	0.48	1.35	0.31	0.63
								290	16	0.30	1.29	0.50	0.46
	Fibre	40.40	7.38	21.2 - 55.0	2144	0.98	1.02						
Molasses	Sucrose	42.70	9.22	31.4 - 77.4	1356	0.99	1.06	19	16	0.98	1.08	2.61	0.98
	Dry substance	42.70	1.98	66.6 - 92.1	1355	0.97	0.34	19	16	0.80	0.68	0.29	0.83
	Brix	83.50	5.12	31.1 - 99.4	1358	0.98	0.80						
	Pol	44.70	5.12	26.8 - 81.1	997	0.99	1.04						
	Reducing sugars	12.20	2.67	8.2 - 19.4	355	0.96	0.53						
	Ash	13.20	1.66	5.4 - 15.3	317	0.99	0.19						
Raw sugar	Pol	98.99	0.39	95.1 - 99.8	4821	0.97	0.07	143	16	0.53	0.06	0.02	0.90
	Moisture	0.30	0.12	0.06 - 1.42	4189	0.97	0.02	143	16	0.78	0.02	-0.02	0.96
	Ash	0.23	0.09	0.05 - 0.77	2346	0.96	0.02	143	16	0.09	0.03	0.00	0.32
	Colour	1587	496	195 - 4014	2313	0.98	94.00	143	16	0.12	119	-108	0.31
	Reducing sugars	0.25	0.10	0.03 - 0.80	1990	0.94	0.02	143	16	0.55	0.02	0.03	0.66
Juice and syrup	Brix	45.55	27.80	0.5 - 78.6	5175	1.00	0.60						
	Pol	42.63	22.90	0.6 - 74.1	3147	1.00	0.56						
	Pol reading	31.91	27.17	2.4 - 94.8	2523	1.00	0.60						
	Ash	4.90	2.88	0.1 - 11.9	1734	0.99	0.24						
	Reducing sugars	0.19	0.22	0.04 - 2.01	1577	0.95	0.05						
Massecuite and magma	Brix	94.10	5.00	81.4 - 106.2	380	0.99	0.56						
	Pol	60.40	16.25	33.3 - 87.0	643	1.00	0.82						
	Dry substance	85.80	4.24	72.0 - 98.2	1729	0.99	0.47						
	Sucrose	72.70	7.98	34.4 - 88.0	2123	0.99	0.91						
	Ash	13.59	0.73	12.0 - 15.2	70	0.90	0.23						
	Reducing sugars	9.92	1.08	8.2 - 13.0	69	0.88	0.38						
	Crystal Content	43.10	6.95	30.0 - 55.6	68	0.89	2.3						
Mill mud	Moisture	74.90	3.29	67.0 - 82.9	637	0.97	0.60						
	Pol	2.70	1.92	0.2 - 9.5	1373	0.97	0.32						
	Fibre	7.90	1.26	4.2 - 14.1	640	0.87	0.45						
	Mud solids	13.80	2.59	8.9 - 19.9	638	0.94	0.64						
	Total insolubles	21.30	2.83	15.4 - 28.0	683	0.96	0.59						

Table 11: Calibration and validation statistics for DA1650 Global 15.2 models cont.

	Constituent	Calibration							Validation			
		Mean	SD	Range	N	R^2	SEC	N	Factors	R^2	SEP	Bias	Slope
Prepared cane	Brix % Juice	19.64	2.98	6.8 - 26.6	3299	0.99	0.31						
	Pol % Juice	16.85	3.40	1.6 - 24.2	3299	0.99	0.33						
	Fibre % Cane	14.30	1.41	10.5 - 21.8	610	0.85	0.54						
	Brix % Cane	16.01	2.40	6.1 - 20.9	3299	0.99	0.28						
	Pol % Cane	13.40	2.68	1.4 - 19.2	3299	0.98	0.28						
	CCS % Cane	12.10	2.14	0.0 - 17.7	3299	0.99	0.35						
	Ash % Cane	1.60	0.82	0.46 - 8.05	743	0.91	0.24						
	Dry matter % Cane	31.66	2.92	23.9 - 39.1	3202	0.99	0.35						
	Moisture % Cane	68.34	2.92	60.9 - 76.1	3202	0.99	0.35						
	Pol in Open Cells	88.58	4.10	80.7 - 99.1	935	0.82	1.7						

SD: standard deviation, N: number of samples, R^2: coefficient of determination, SEC: standard error of calibration, SEP: standard error of prediction

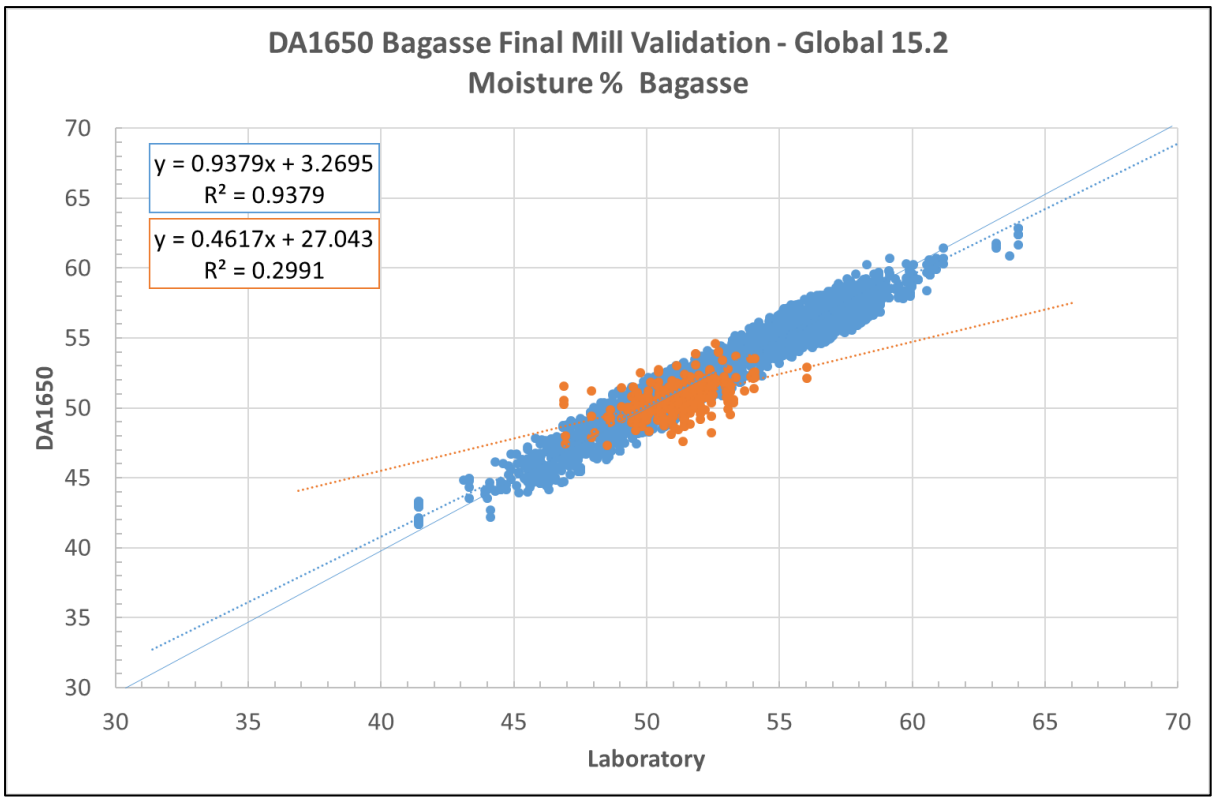

Figure 25: Calibration (blue) and validation (orange) of Global 15.2 bagasse moisture final mill model

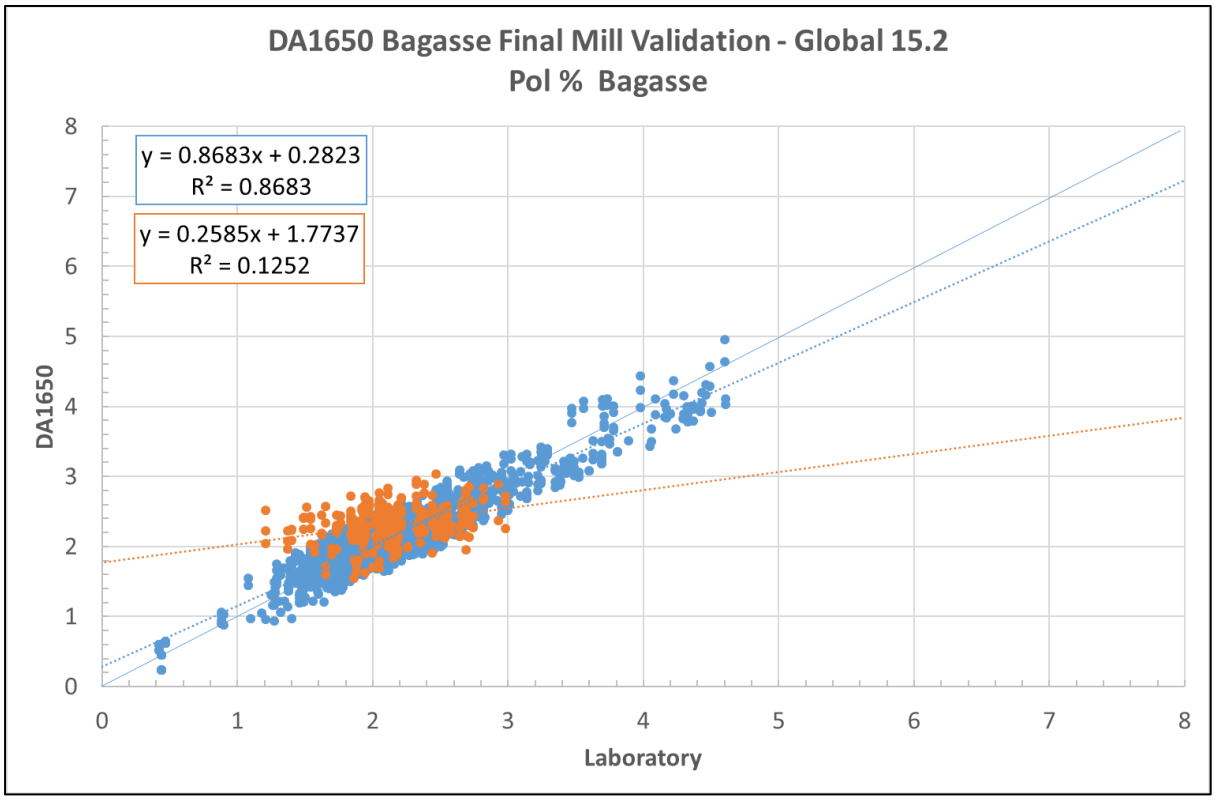

Figure 26: Calibration (blue) and validation (orange) of Global 15.2 bagasse pol final mill model

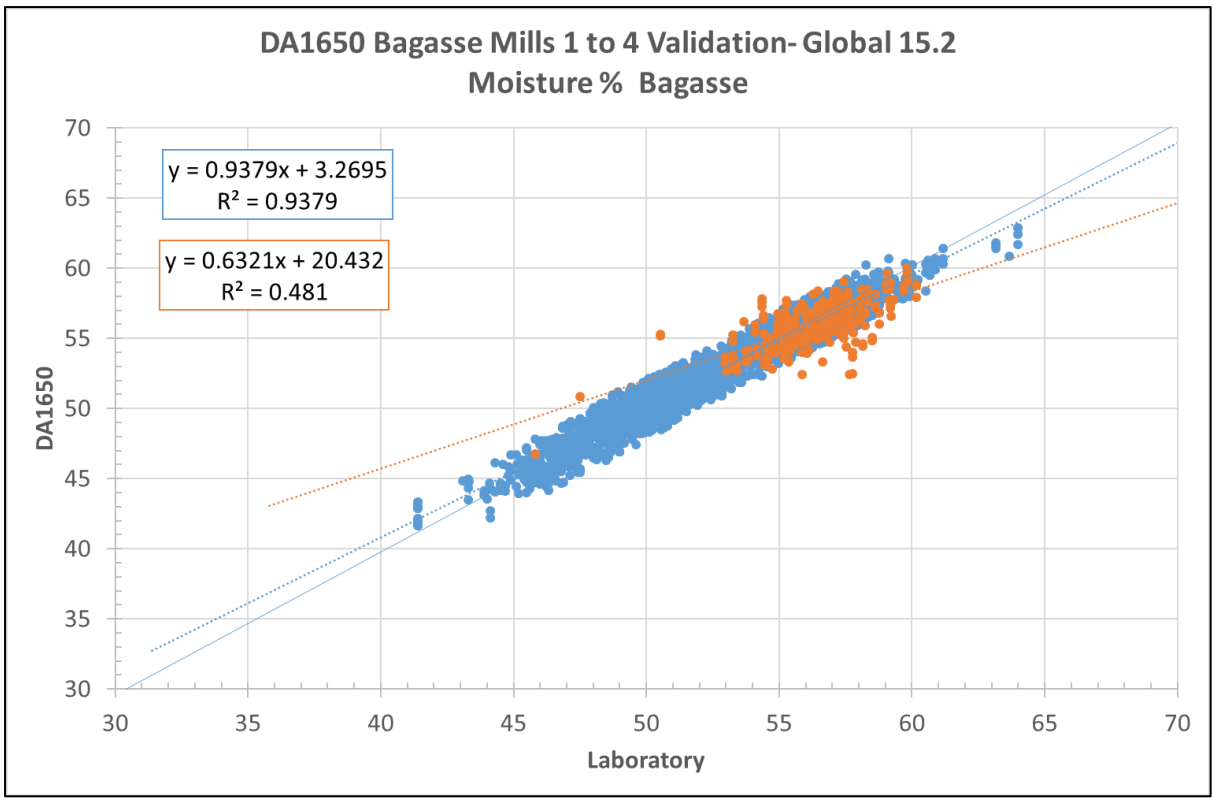

Figure 27: Calibration (blue) and validation (orange) of Global 15.2 bagasse moisture primary mill model

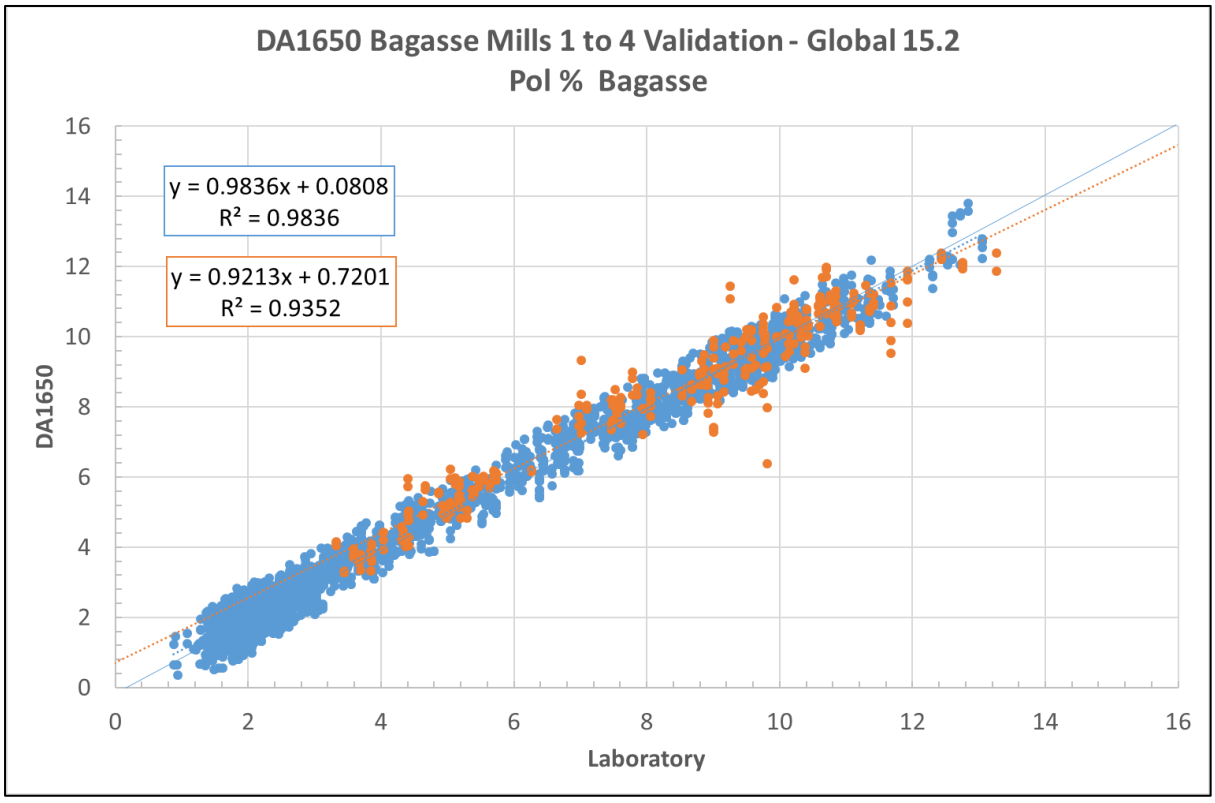

Figure 28: Calibration (blue) and validation (orange) of Global 15.2 bagasse pol primary mill model

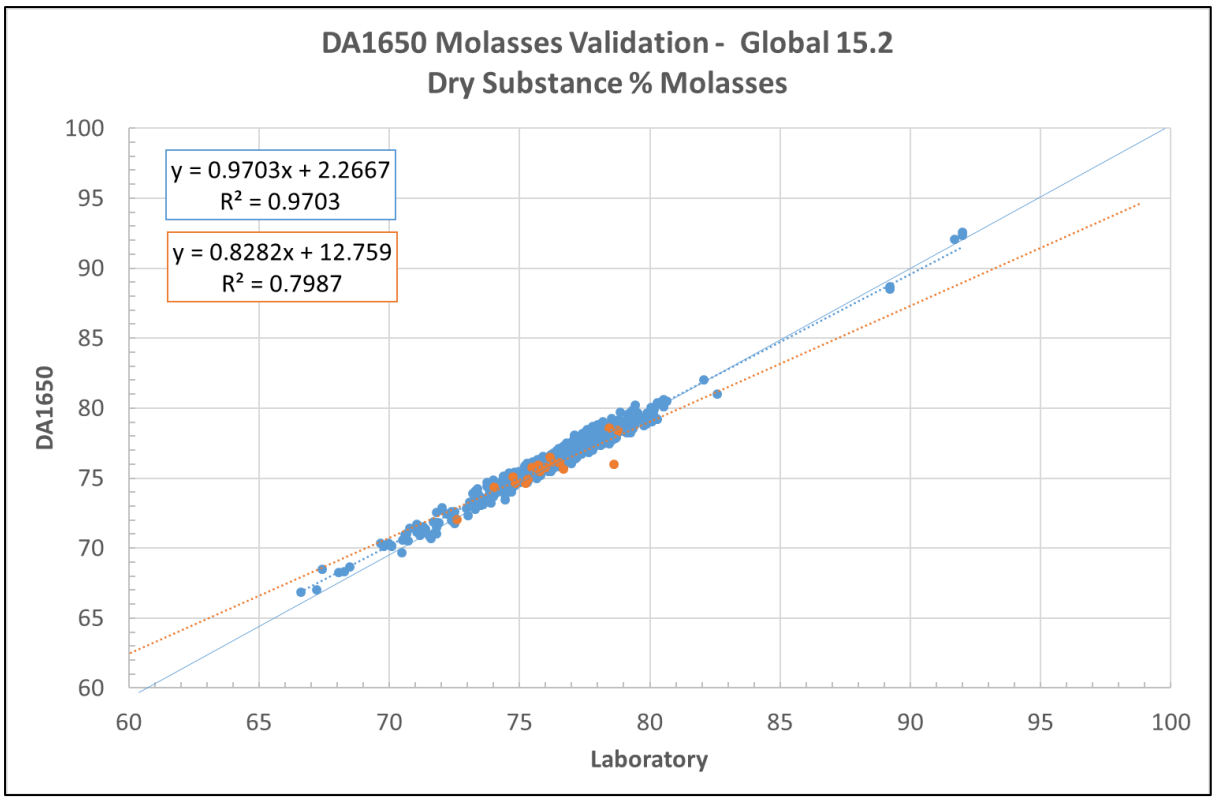

Figure 29: Calibration (blue) and validation (orange) of Global 15.2 molasses dry substance model

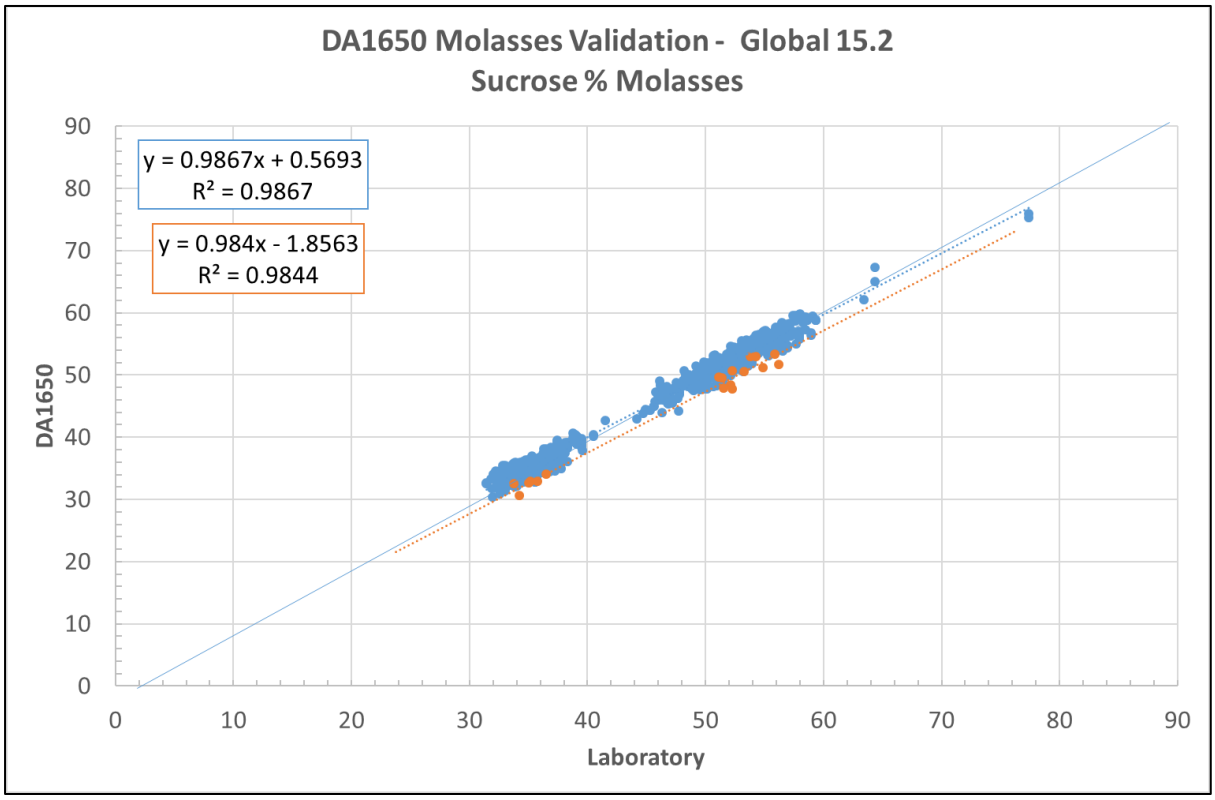

Figure 30: Calibration (blue) and validation (orange) of Global 15.2 molasses sucrose model

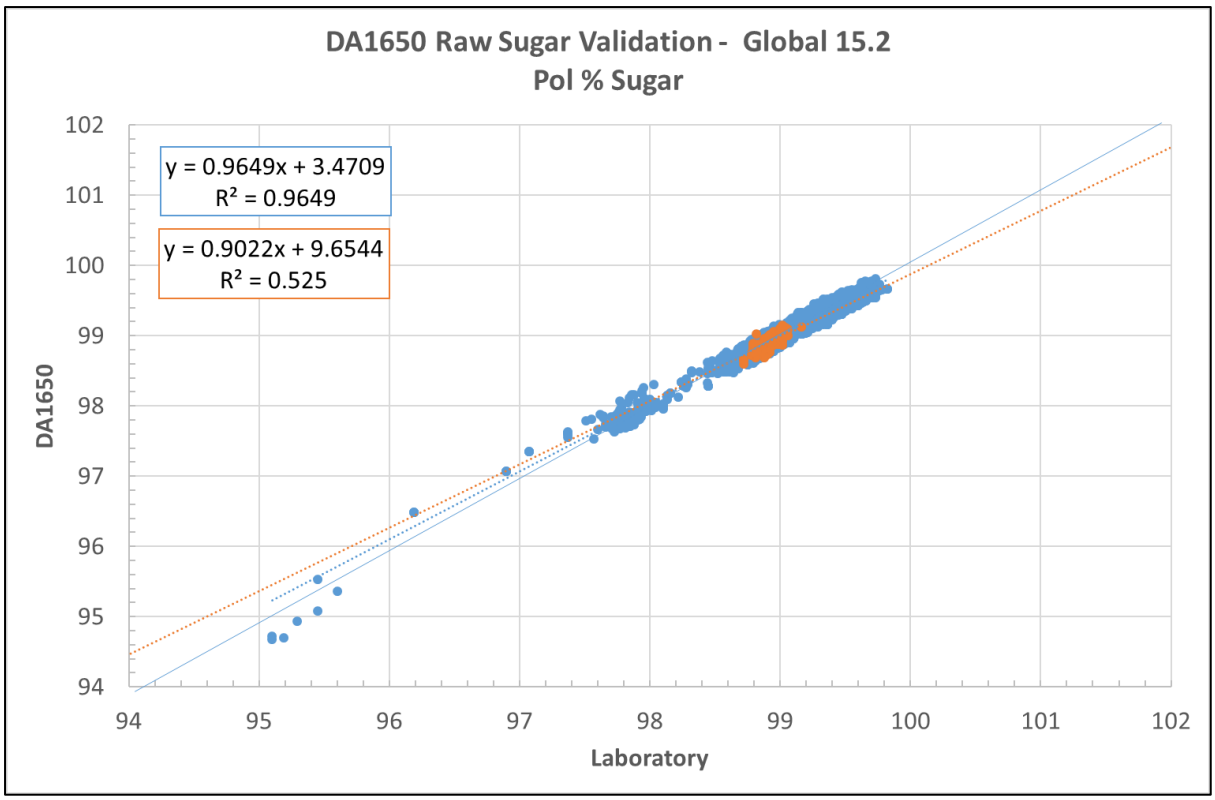

Figure 31: Calibration (blue) and validation (orange) of Global 15.2 sugar pol model

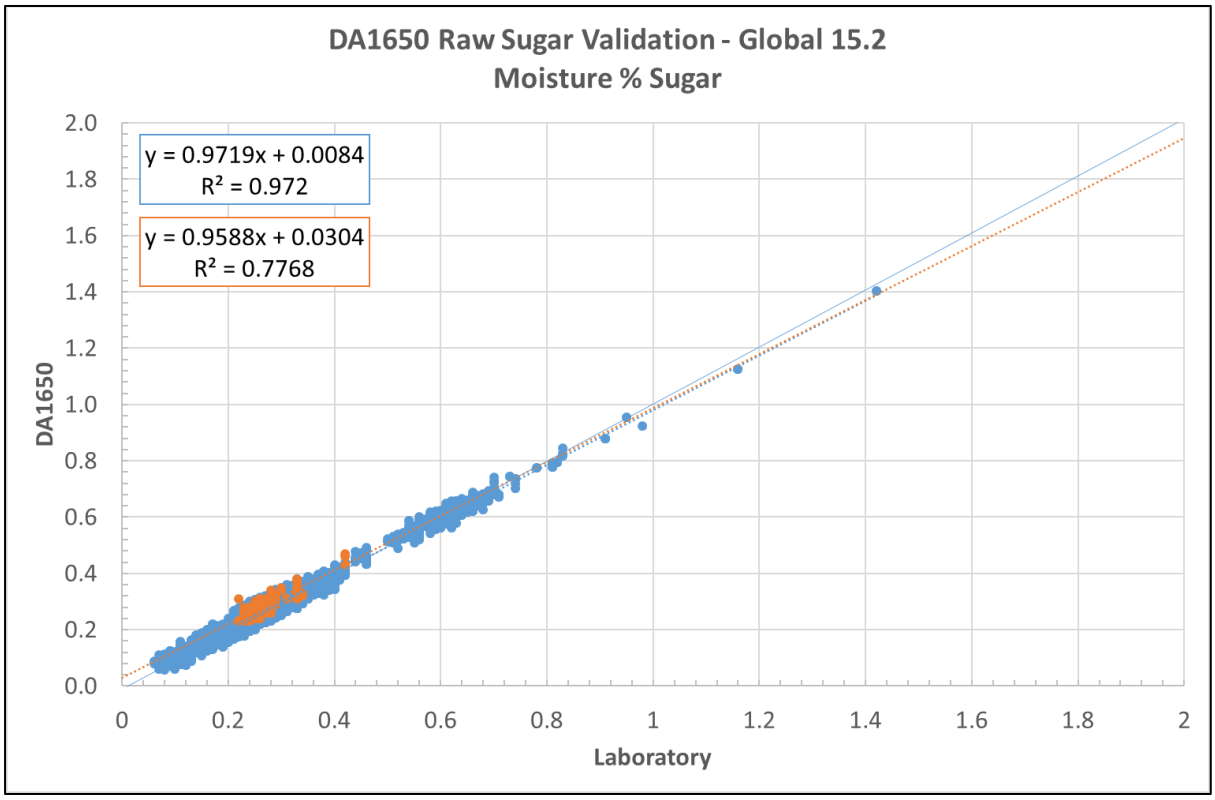

Figure 32: Calibration (blue) and validation (orange) of Global 15.2 sugar moisture model

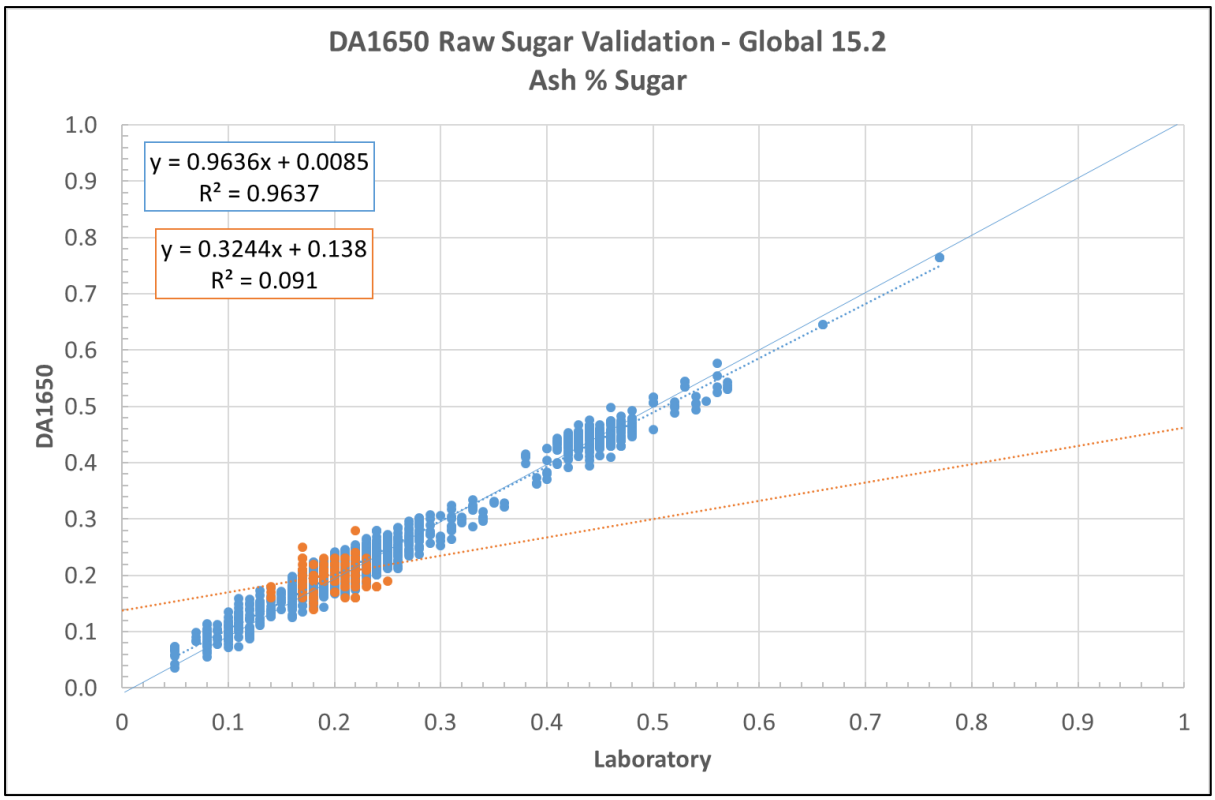

Figure 33: Calibration (blue) and validation (orange) of Global 15.2 sugar ash model

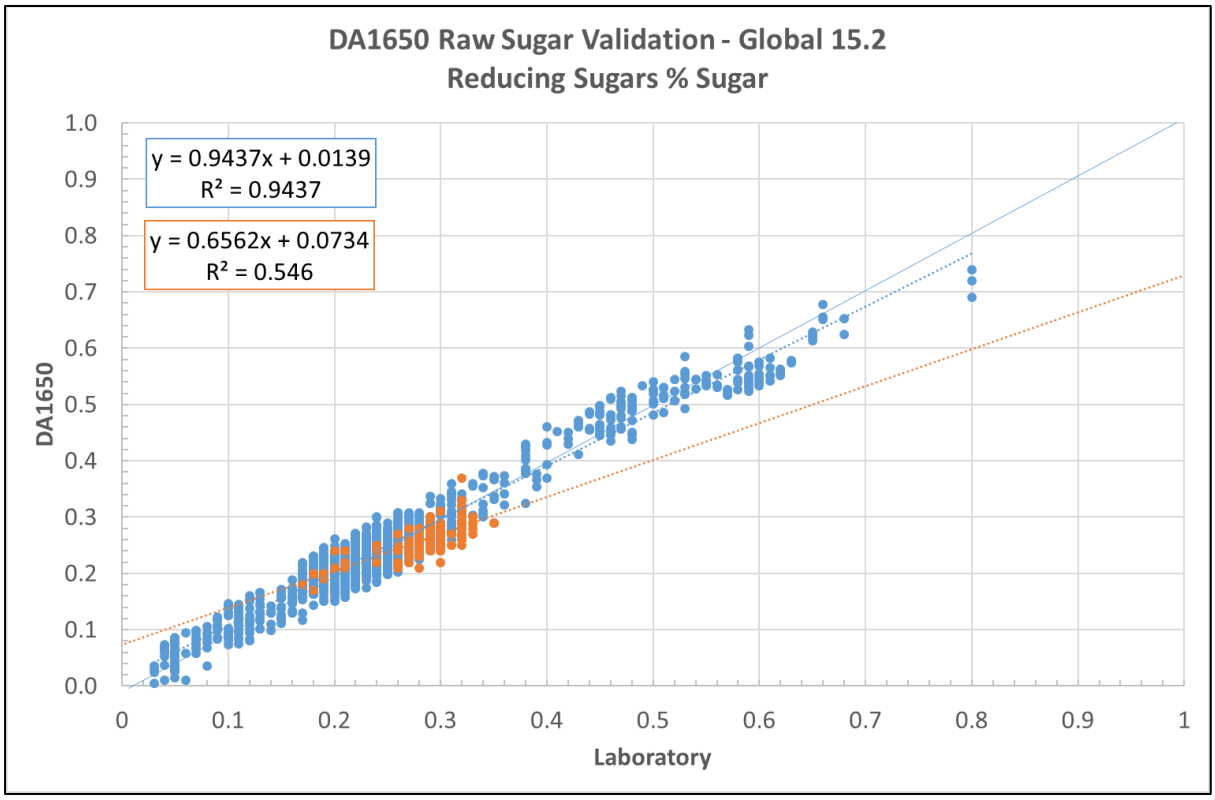

Figure 34: Calibration (blue) and validation (orange) of Global 15.2 sugar reducing sugars model

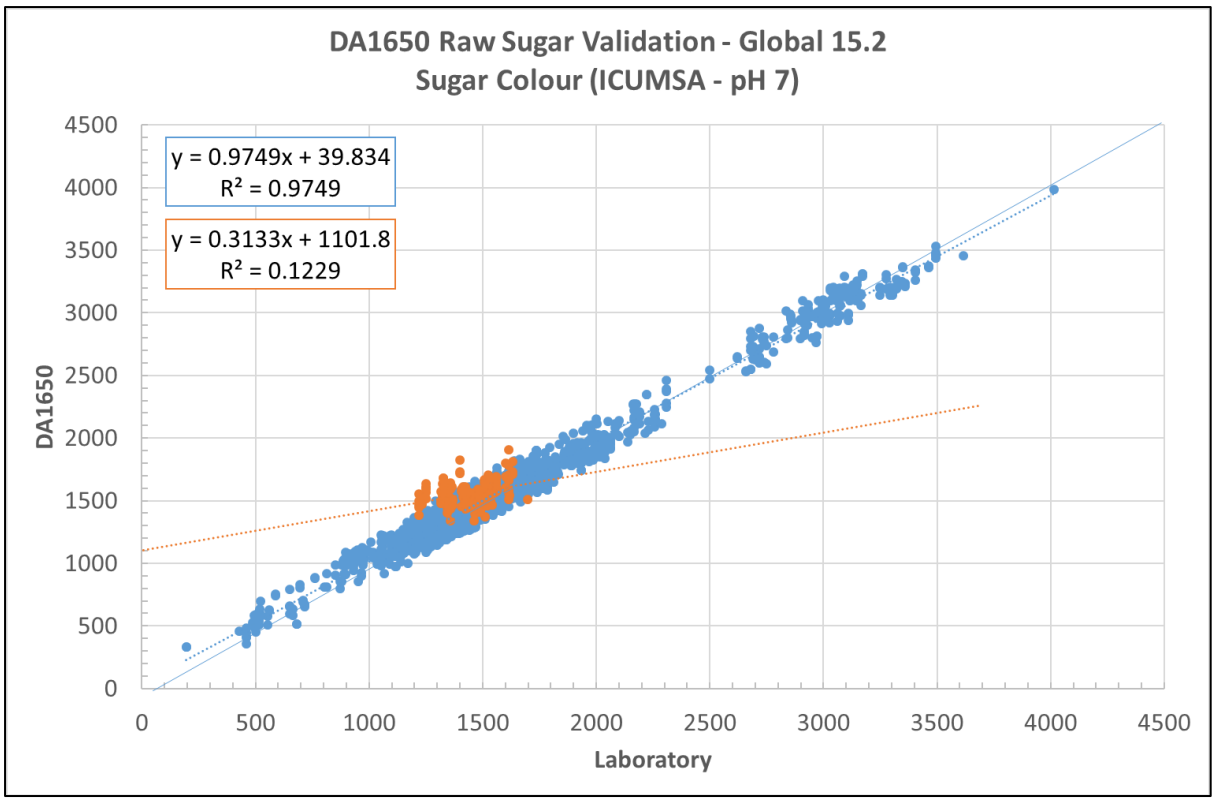

Figure 35: Calibration (blue) and validation (orange) of Global 15.2 sugar colour model

6.1.3.4. Global 16.1

The Global 16.1 models were developed and loaded onto all instruments in November of 2016. The SRA instrument was installed in Mill 2 until the end of the season. The trial at Mill 2 was very successful from a data collection perspective. The mill management and laboratory staff were committed to sample analysis by NIR and provided extra staffing where required. Additionally, a large number of samples were set aside in the weeks prior to the trial, which were analysed by SRA staff following installation of the instrument. The Mill 2 staff were particularly focussed on the performance of the DA1650 for raw sugar and the pan products: liquor, massecuite, magma and molasses. Mill 1 also conducted routine analysis of molasses and massecuites on the DA1650, in addition to raw sugar. This was the first time that there was a large amount of data available for the pan products, which was useful and demonstrated a significant problem.

The calibration statistics for each model are provided in Table 12 and the validation statistics for each mill across the 2016 and 2017 seasons are provided in Table 13. The associated calibration/ validation plots are provided in Figure 36 to Figure 63.

Both Mill 1 and Mill 2 showed large amounts of scatter in the validation data for one or more constituents in each pan product. The scatter was rarely consistent between mills and was not typically restricted to a single product sub-type, e.g. B molasses. The Mill 1 massecuite dry substance plot (Figure 36) shows suitable predictions for products with a dry substance over 87 %, but poor predictions for many samples with a dry substance less than 87 %. Alternatively, the Mill 2 plot in Figure 38 shows the majority of samples analysed had a dry substance greater than 87 % and that there was a relatively tight core of samples, surrounded by a large number of samples with poor correlation between the predicted and reference values.

The molasses plots from Mill 2 showed a similar response (Figure 44 and Figure 45), with both demonstrating that the majority of samples showed a good relationship between the predicted and reference values, but a subset of samples presented with significant scatter about the regression line. The sample plots from Mill 1 showed very nice relationships between the predicted and reference values for both dry substance (Figure 42) and sucrose (Figure 43). This may be because the model is working effectively for Mill 1, or may be that the validation sample size was too small to see the same variability observed at Mill 2, as it is only occurring for a small percentage of the population.

The scatter is consistent in its distribution about the regression line, showing little bias, and the slope of the validation data is typically close to 1. This suggests the scatter is not due to systematic deviation of the NIR predicted values from the reference values, nor directly associated with reference lab error or NIR system error. Rather, it suggests that a subset of samples are not well represented in that calibration set. Consequently, they are not predicted well.

Prior to re-calibrating to include this type of sample, we must evaluate whether it is a 'desired' sample type. Including samples that are different and rare may increase the error of the predictions for the normal and frequent samples. We must evaluate if these samples are typical, or not.
An example of this problem is the prediction of cane payment parameters for rotting cane by the CAS. The cane is still cane, and mostly looks like cane, spectrally. However, slight spectral variations due to the change in composition mean that the standard CAS calibration models do not predict these samples well. As rotting is (hopefully), rare and not very repeatable, building this data into the CAS calibrations to allow prediction of rotting cane increases the error of all of the models, and therefore increases the error for standard predictions.

The samples with high scatter do not always have GH or nH values greater than the 3.0 and 0.8 thresholds, respectively, although occasionally they do. This indicates that they are spectrally similar to a 'normal' sample. Discussion with mills staff identified that the cane supply was 'odd' in 2016, particularly with relation to dextran levels. In both the Mill 1 and Mill 2 regions, the dextran levels were much higher than normal. During this period, one on the mills indicated that their HPLC was requiring more frequent maintenance due to column clogging, which may have been due to the dextran.

The G16.1 models were continued into the 2017 season to determine whether their poor performance for the pan products was due to abnormal cane supply (Figure 40 to Figure 41 and Figure 46 to Figure 47). Although the massecuite looked promising, the molasses samples showed large amounts of scatter, indicating the effect was not seasonal and unlikely to be dextran, which had reduced to normal levels in the 2017 season.

In both 2016 and 2017 the sucrose model for juice showed two distinct groupings in the syrup samples from Mill 2 (Figure 49 and Figure 51). Both were relatively linear, with a slope close to 1, but one of the groups had a strong bias; the NIR spectroscopy system was under-predicting by approximately 5 units.

The pan products were re-calibrated and the G17.1 models are described in Section 6.1.3.5.

In 2016 the G16.1 raw sugar models performed well for both mills, although the Mill 1 models showed bias adjustments were required for most constituents (Figure 52 to Figure 61). Both the dextran and starch models fail to quantify the varying concentration in the samples, with regression lines near-horizontal for both.

Table 12: Calibration statistics for Global 16.1 calibration models

	Constituent name	Calibration statistics								
		SEC	R^2	Mean	SD	Min	Max	N	ECL	Fact.
Raw sugar	Pol % Sugar	0.09	0.93	99.03	0.32	97.44	99.99	9313	0.10	15
	Pol - low purity % Sugar	0.09	0.98	98.93	0.74	92.09	99.99	9716	0.11	16
	Moisture % Sugar	0.03	0.90	0.29	0.09	0.06	1.06	5967	0.03	13
	Ash % Sugar	0.02	0.84	0.21	0.05	0.01	0.66	3912	0.02	15
	Colour (ICUMSA)	171	0.95	1615	754	10	6476	4947	205	14
	Reducing Sugars % Sugar	0.03	0.98	0.26	0.23	0.03	1.99	4767	0.04	15
	Fine Grain %	2.25	0.71	14.41	4.11	4.00	30.00	4145	2.69	12
	Filtrability	3.40	0.76	73.97	7.01	51.00	90.00	4216	4.08	15
	Starch (ppm)	11.41	0.10	49.69	12.02	19.00	80.00	4295	13.69	5
	Dextran (ppm)	29.84	0.01	44.93	22.50	0.00	99.00	454	35.81	1
Bagasse	Pol % Bagasse- Primary mills	0.49	0.98	5.29	3.35	0.42	13.05	5530	0.59	13
	Pol % Bagasse- Final mill	0.38	0.89	2.67	1.12	0.42	5.94	3232	0.45	12
	Fibre % Bagasse	1.25	0.97	38.83	7.47	12.10	55.02	2851	1.50	11
	Moisture % Bagasse	1.07	0.90	52.93	3.41	41.40	63.99	8153	1.28	11
	Brix % bagasse	0.06	0.37	1.32	0.07	1.18	1.42	15	0.07	1
Juice and syrup	Brix % Juice/Syrup	0.73	1.00	43.75	28.38	0.10	78.60	10976	0.88	14
	Pol % Juice/Syrup	0.63	1.00	30.99	24.96	0.00	73.20	6765	0.75	16
	Pol Reading (°Z)	0.67	1.00	34.31	27.20	2.04	94.82	5667	0.81	9
	Ash % Juice/Syrup	0.24	0.99	5.03	2.85	0.12	11.88	1689	0.29	12
	Reducing Sugars % Juice/Syrup	0.07	0.92	0.19	0.23	0.02	3.40	1741	0.08	11
	CCS	1.01	0.71	13.15	1.88	9.77	16.36	30	1.21	3
	Sucrose % Juice/Syrup	1.70	0.52	60.34	2.44	55.40	69.10	81	2.03	2
	Dry substance % Juice/Syrup	0.51	0.92	67.83	1.82	63.80	71.40	72	0.62	3
Massecuite and magma	Brix % Massecuite	0.64	0.99	93.18	5.98	68.20	101.7	1575	0.77	14
	Pol % Massecuite	1.21	0.99	67.35	14.72	31.90	87.17	1581	1.45	16
	Dry Substance % Massecuite	0.47	0.72	86.02	4.56	72.62	94.70	1843	0.57	12
	Sucrose % Massecuite	1.00	0.93	71.64	9.03	35.56	88.00	2344	1.20	15
	Ash % Massecuite	0.16	0.95	13.68	0.70	12.05	15.34	135	0.19	10
	Reducing Sugars % Massecuite	0.27	0.94	9.95	1.10	8.16	13.01	142	0.32	10
	Crystal % Massecuite	2.55	0.86	43.12	6.90	30.00	55.60	69	3.05	6
	Massecuite Impurity:Water	0.10	0.98	0.92	0.71	0.26	4.56	1190	0.12	12
	Water % Massecuite	0.53	0.85	14.08	4.69	5.30	27.96	1266	0.64	10
Molasses	Brix % Molasses	0.94	0.98	82.51	7.55	12.40	99.40	1800	1.13	12
	Pol % Molasses	1.44	0.98	44.53	10.65	26.37	81.14	1578	1.72	14
	Dry Substance % Molasses	0.26	0.97	76.43	1.62	68.28	92.01	1955	0.31	13
	Sucrose % Molasses	1.22	0.97	41.92	8.97	31.43	92.34	2580	1.47	16
	Ash % Molasses	0.30	0.82	13.40	0.71	11.18	15.34	365	0.36	8
	Reducing Sugars % Molasses	0.86	0.88	12.21	2.50	8.16	17.31	514	1.03	8
Mill mud	Moisture % Mud	1.07	0.89	75.87	3.52	67.00	82.85	871	1.29	7
	Pol % Mud	0.47	0.94	2.60	1.91	0.05	9.45	1875	0.56	11
	Fibre % Mud	0.63	0.56	7.74	0.94	4.71	10.71	814	0.75	10
	Mud Solids % Mud	1.22	0.71	13.77	2.49	8.52	19.86	758	1.46	5
	Total Insolubles % Mud	1.31	0.78	21.44	2.81	14.94	28.00	832	1.57	7

Table 12: Calibration statistics for Global 16.1 calibration models cont.

	Constituent name	Calibration statistics								
		SEC	R^2	Mean	SD	Min	Max	N	ECL	Fact.
Prepared cane	Brix % Juice	0.33	0.99	19.57	2.70	7.25	25.60	6415	0.39	13
	Pol % Juice	0.40	0.98	16.76	3.01	2.58	23.50	6203	0.49	15
	Fibre % Cane	0.59	0.86	15.25	1.61	10.31	24.58	5113	0.71	13
	Brix % Cane	0.29	0.98	16.04	2.27	6.51	21.07	6322	0.35	14
	Pol % Cane	0.35	0.98	13.39	2.44	2.24	18.87	6261	0.42	14
	CCS % Cane	0.43	0.97	12.06	2.56	0.06	17.80	6227	0.5	14
	Ash % Cane	0.31	0.79	1.57	0.69	0.46	10.52	5375	0.38	14
	DryMatter % Cane	0.51	0.95	31.93	2.21	24.33	39.82	4911	0.6	1
	Moisture % Cane	0.51	0.95	68.07	2.22	60.19	75.67	4920	0.6	14
	Pol in Open Cells (POC)	0.50	0.88	89.10	1.46	78.89	94.30	5755	0.6	15

SD: standard deviation, N: number of samples, R^2: coefficient of determination, SEC: standard error of calibration, ECL: error control limit, Fact.: number of factors

Table 13: Validation statistics for Global 16.1 calibration models

	Constituent name	Validation statistics Mill 2 2016				Validation statistics Mill 2 2017				Validation statistics Mill 1 2016			
		N	R^2	Bias	SEP	N	R^2	Bias	SEP	N	R^2	Bias	SEP
Raw sugar	Pol % Sugar	83	0.56	-0.11	0.12					118	0.61	0.25	0.07
	Pol - low purity % Sugar									118	0.65	0.27	0.07
	Moisture % Sugar	83	0.78	0.03	0.03					118	0.47	-0.03	0.02
	Ash % Sugar	83	0.38	0.04	0.03					118	0.26	-0.04	0.02
	Colour (ICUMSA)	83	0.21	151	323					118	0.33	-103	124
	Reducing Sugars % Sugar	83	0.54	-0.02	0.04					118	0.73	0.08	0.02
	Fine Grain % Sugar	83	0.04	2.80	3.92					118	0.59	3.12	1.65
	Filtrability	83	0.27	0.40	4.23					118	0.23	5.02	2.57
	Starch (ppm)	83	0.00	2.33	14.30					118	0.00	-9.00	6.97
	Dextran (ppm)	83	0.00	-70.98	38.01					118	0.00	-33	25
Bagasse	Pol % Bagasse- PM												
	Pol % Bagasse- FM												
	Fibre % Bagasse												
	Moisture % Bagasse												
	Brix % bagasse												
Juice and syrup	Brix % Juice												
	Pol % Juice												
	Pol Reading (°Z)												
	Ash % Juice												
	Reducing Sugars % Juice												
	CCS												
	Sucrose % Juice	150	0.06	-0.30	4.93	21	0.98	-0.85	3.58				
	Dry substance % Juice	150	0.59	-0.05	1.44	21	0.98	0.37	1.18				

Table 13: Validation statistics for Global 16.1 calibration models cont.

	Constituent name	Validation statistics Mill 2 2016				Validation statistics Mill 2 2017				Validation statistics Mill 1 2016			
		N	R^2	Bias	SEP	N	R^2	Bias	SEP	N	R^2	Bias	SEP
Massecuite and magma	Brix % Massecuite												
	Pol % Massecuite												
	Dry Substance % Mass.	607	0.08	0.33	1.36	18	0.06	-0.52	0.62	284	0.77	-0.27	1.26
	Sucrose % Massecuite	606	0.91	-0.04	3.18	20	0.54	0.30	0.74	254	0.31	-1.87	2.39
	Ash % Massecuite												
	Reducing Sugars % Mass.												
	Crystal % Massecuite												
	Mass. Impurity:Water												
	Water % Massecuite												
Molasses	Brix % Molasses												
	Pol % Molasses												
	Dry Substance % Mol.	469	0.56	-0.06	2.83	92	0.97	0.02	0.84	116	0.56	0.05	0.57
	Sucrose % Molasses	469	0.76	-1.29	3.68	92	0.92	-1.97	3.43	104	0.36	0.71	1.70
	Ash % Molasses												
	Reducing Sugars % Mol.												

N: number of samples, R^2: coefficient of determination, SEC: standard error of calibration, SEP: standard error of prediction, Mol.: molasses, Mass: massecuite, PM: primary mill, FM: final mill

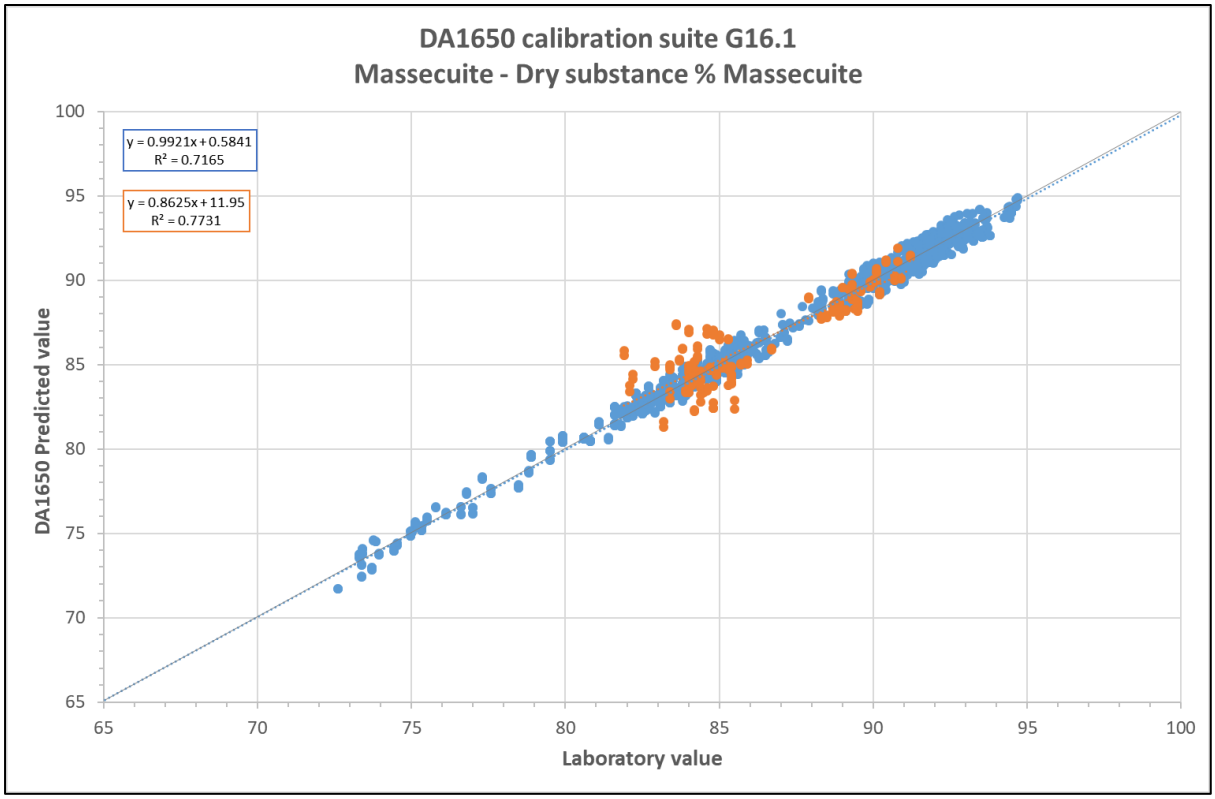

Figure 36: Calibration (blue) and validation (orange) of Global 16.1 massecuite dry substance at Mill 1 in 2016

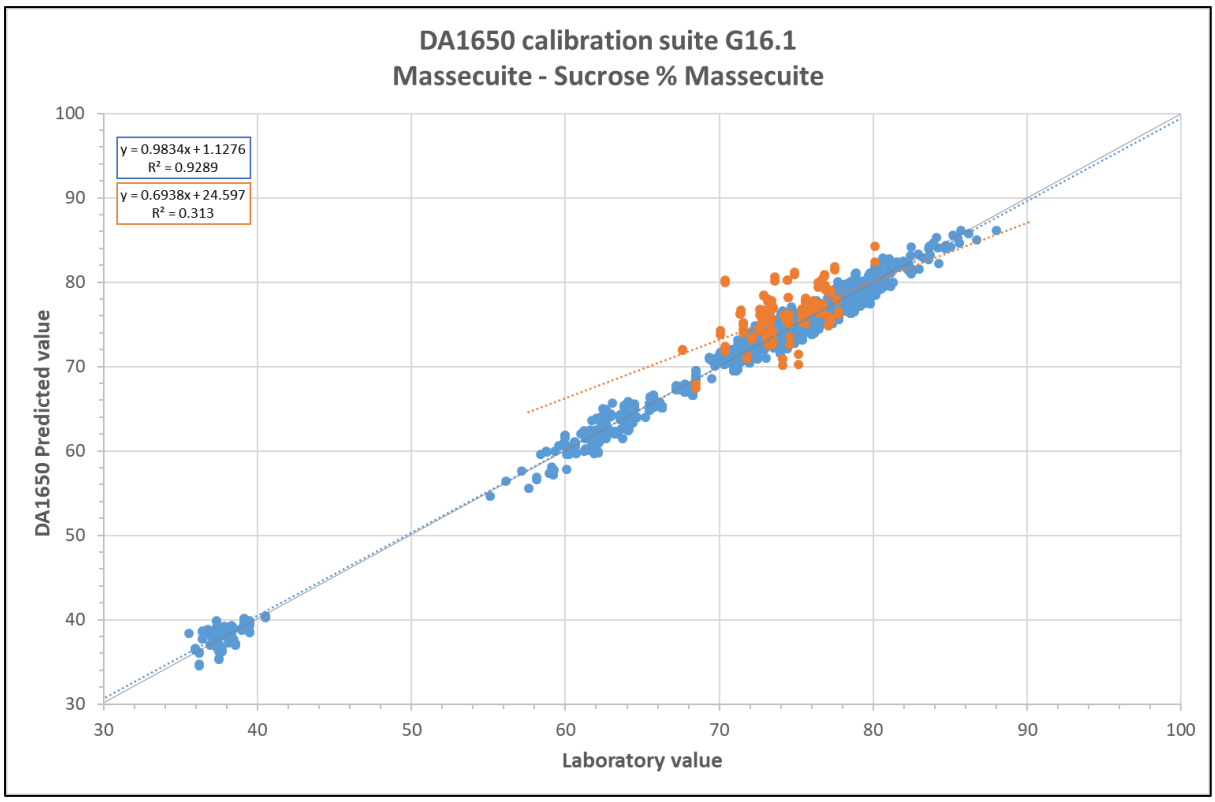

Figure 37: Calibration (blue) and validation (orange) of Global 16.1 massecuite sucrose at Mill 1 in 2016

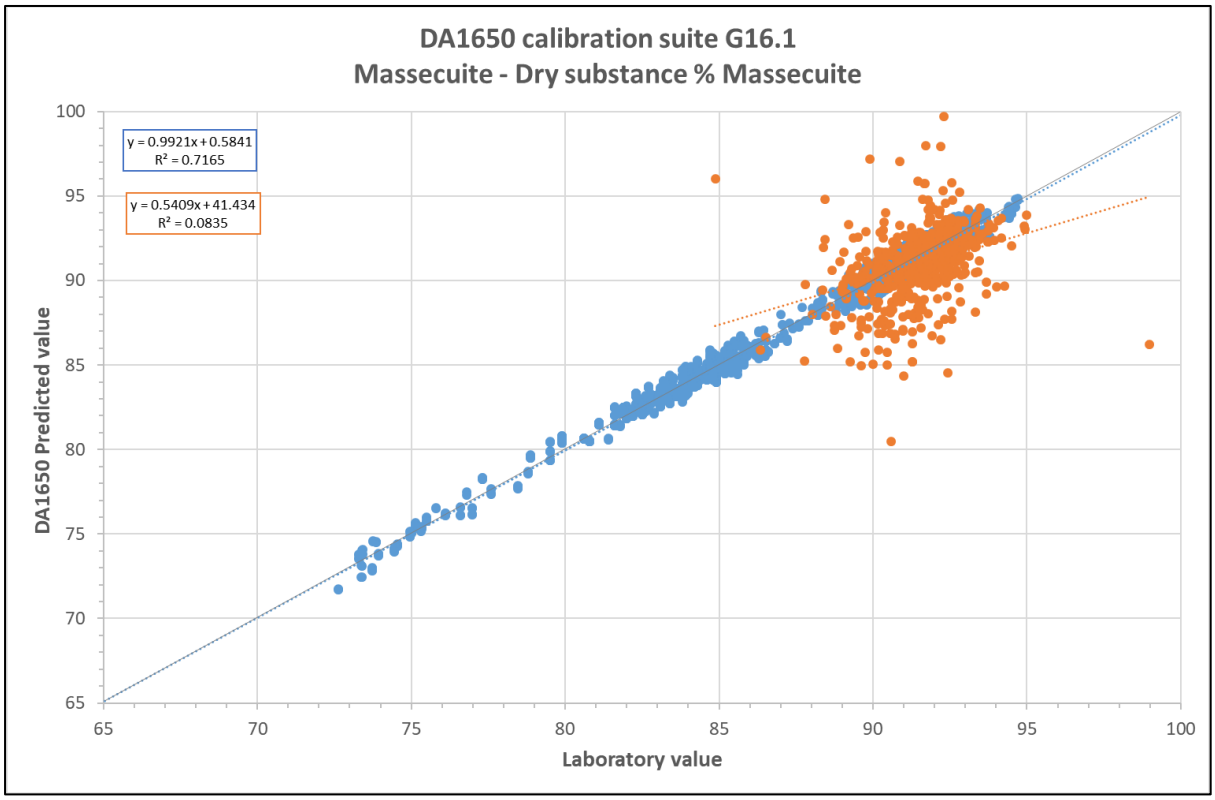

Figure 38: Calibration (blue) and validation (orange) of Global 16.1 massecuite dry substance at Mill 2 in 2016

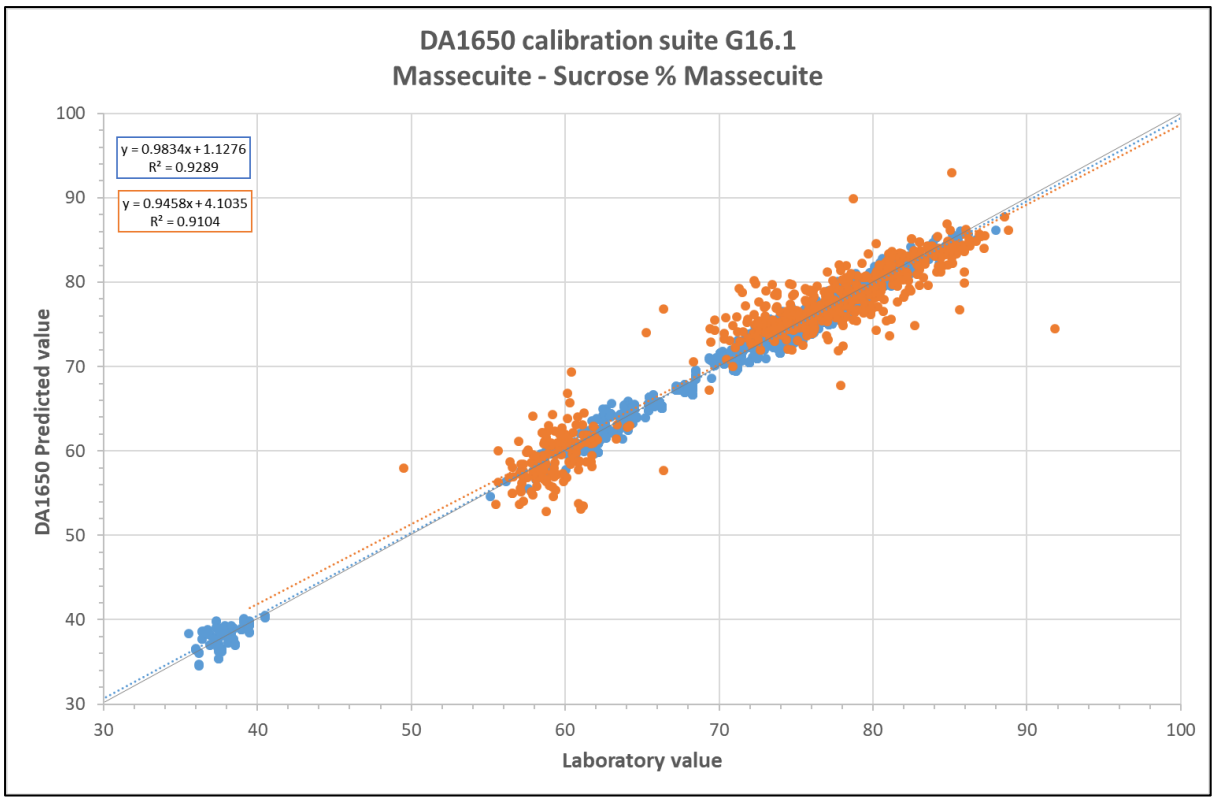

Figure 39: Calibration (blue) and validation (orange) of Global 16.1 massecuite sucrose at Mill 2 in 2016

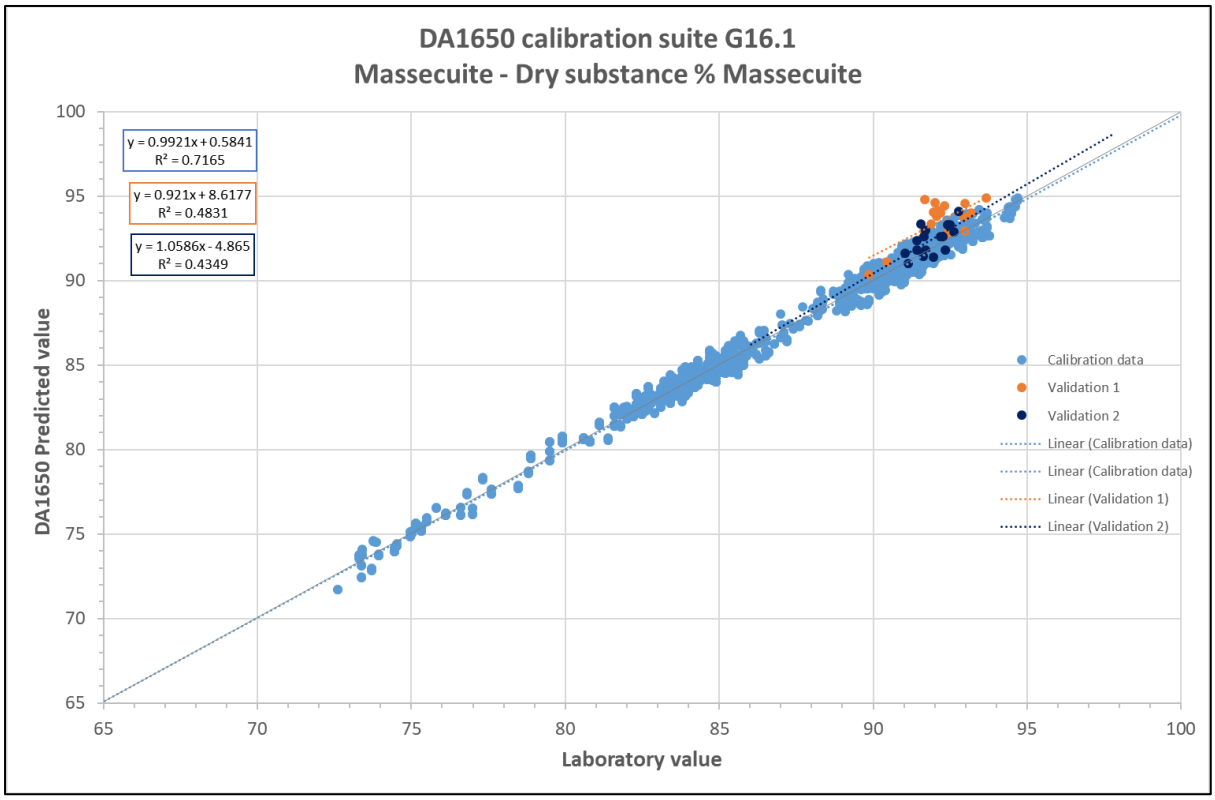

Figure 40: Calibration (blue) and validation (orange and navy) of Global 16.1 massecuite dry substance at Mill 2 in 2017

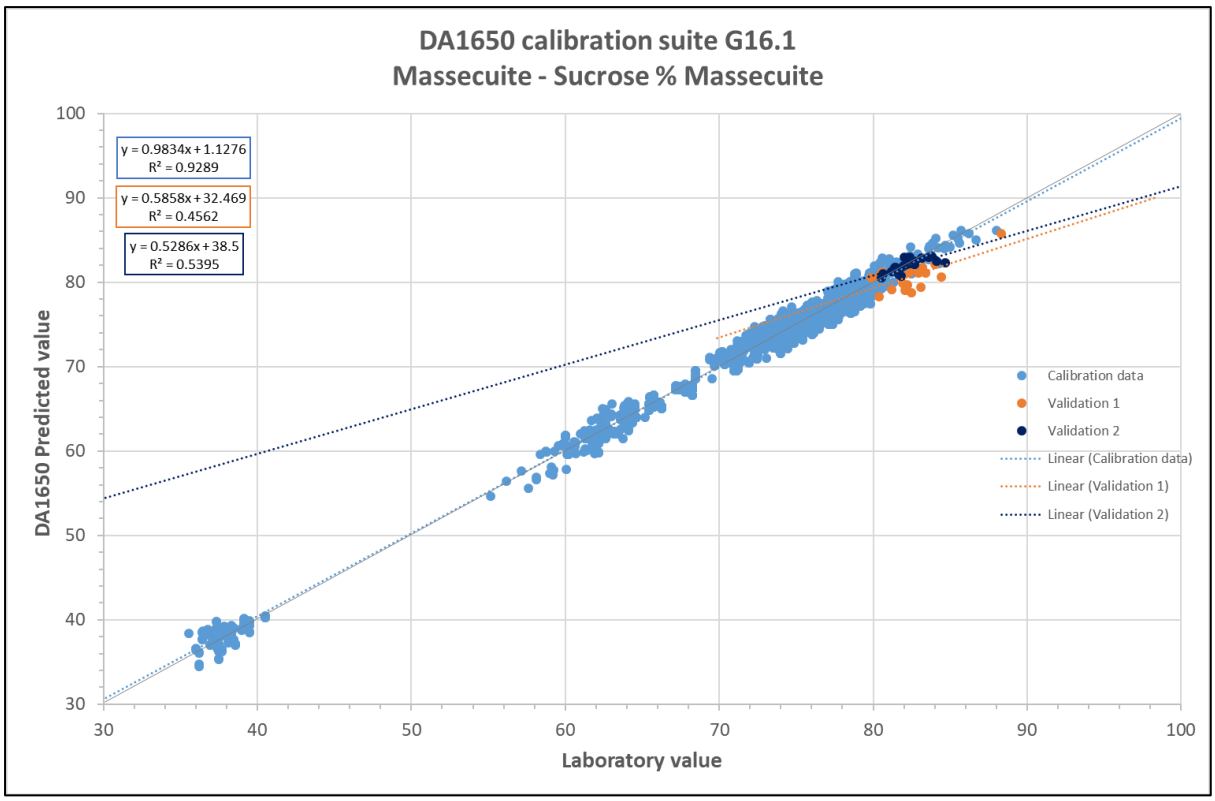

Figure 41: Calibration (blue) and validation (orange and navy) of Global 16.1 massecuite sucrose at Mill 2 in 2017

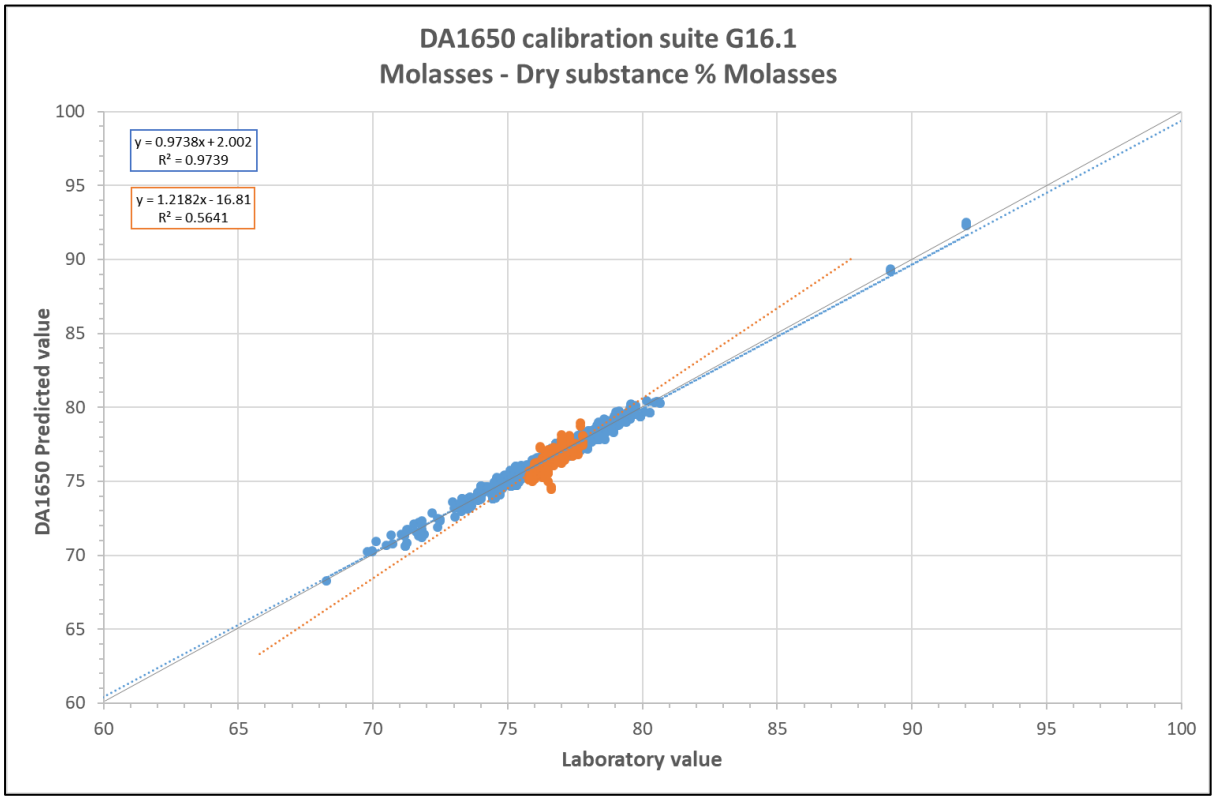

Figure 42: Calibration (blue) and validation (orange) of Global 16.1 molasses dry substance at Mill 1 in 2016

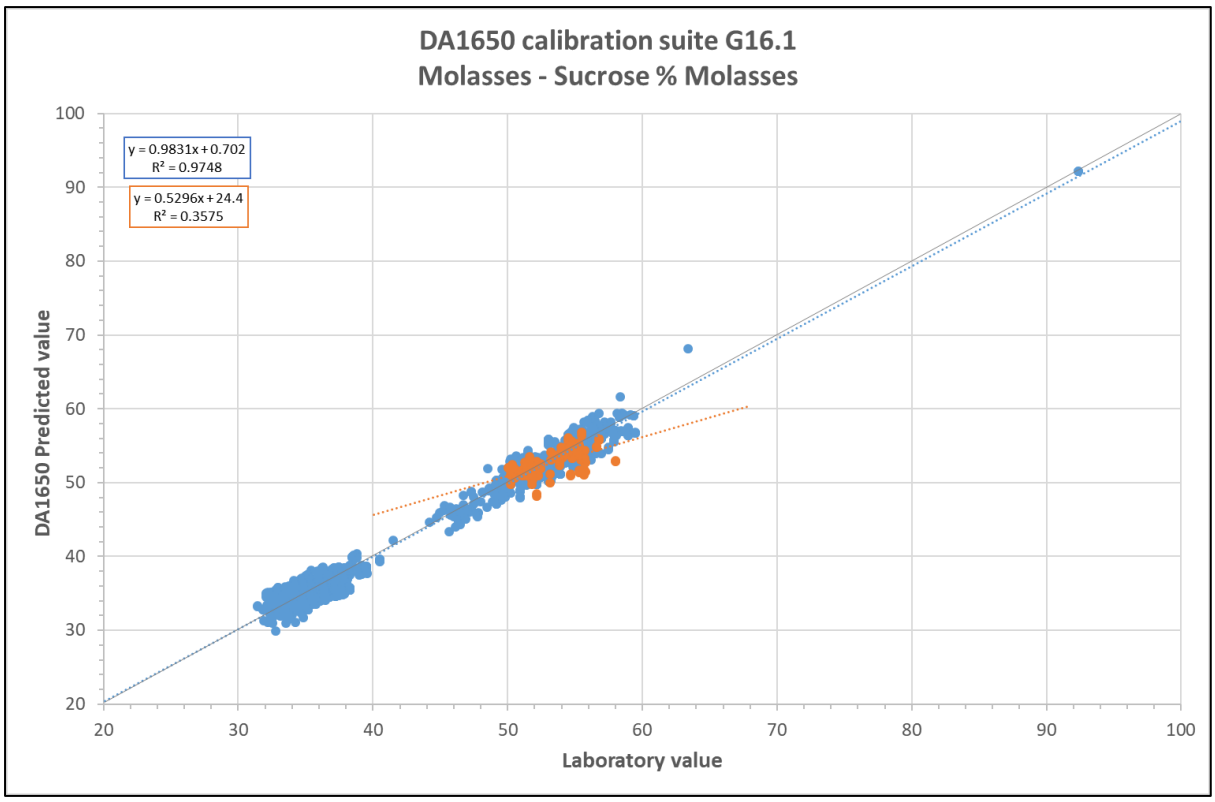

Figure 43: Calibration (blue) and validation (orange) of Global 16.1 molasses sucrose at Mill 1 in 2016

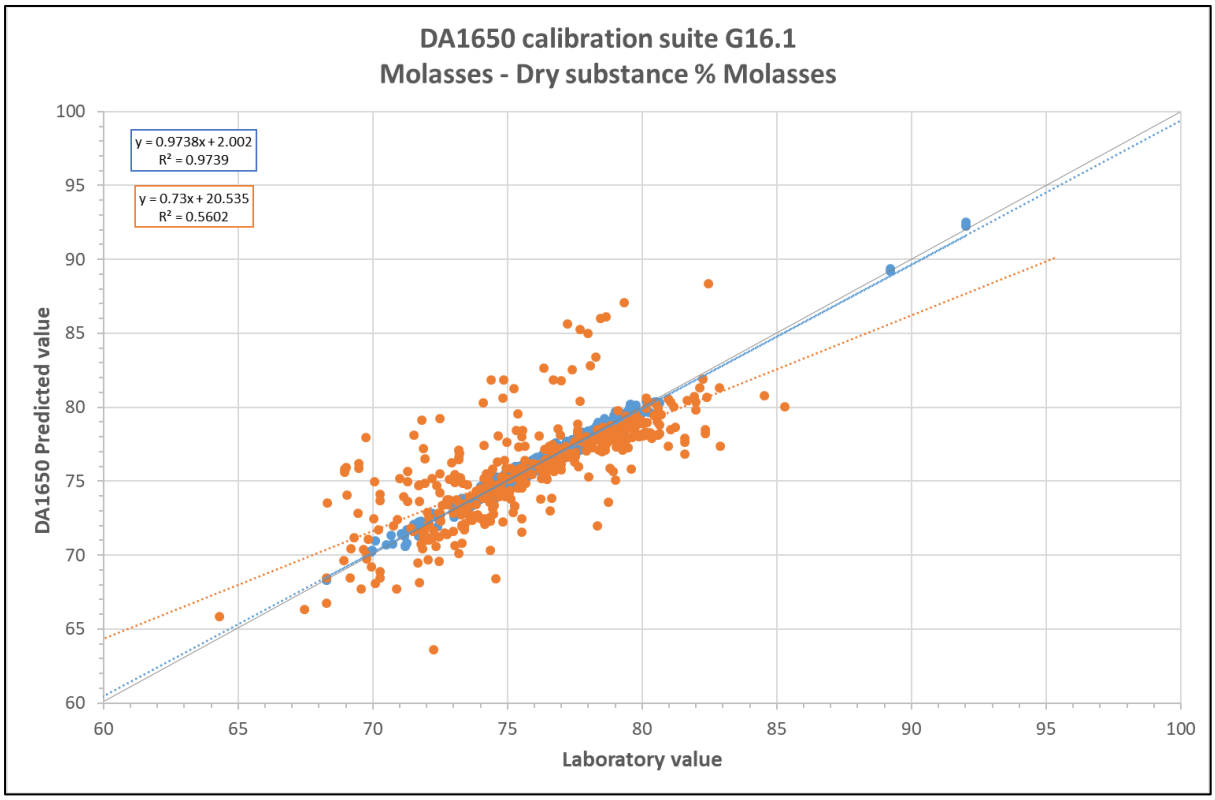

Figure 44: Calibration (blue) and validation (orange) of Global 16.1 molasses dry substance at Mill 2 in 2016

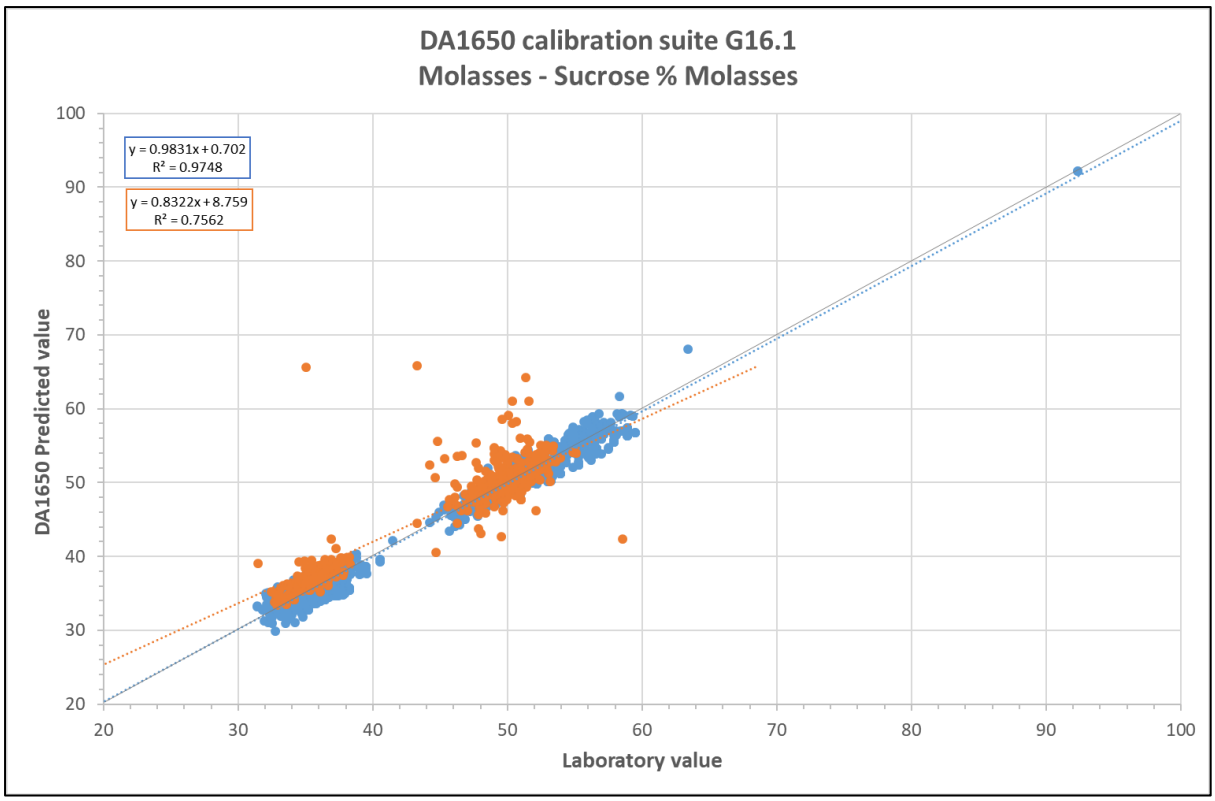

Figure 45: Calibration (blue) and validation (orange) of Global 16.1 molasses sucrose at Mill 2 in 2016

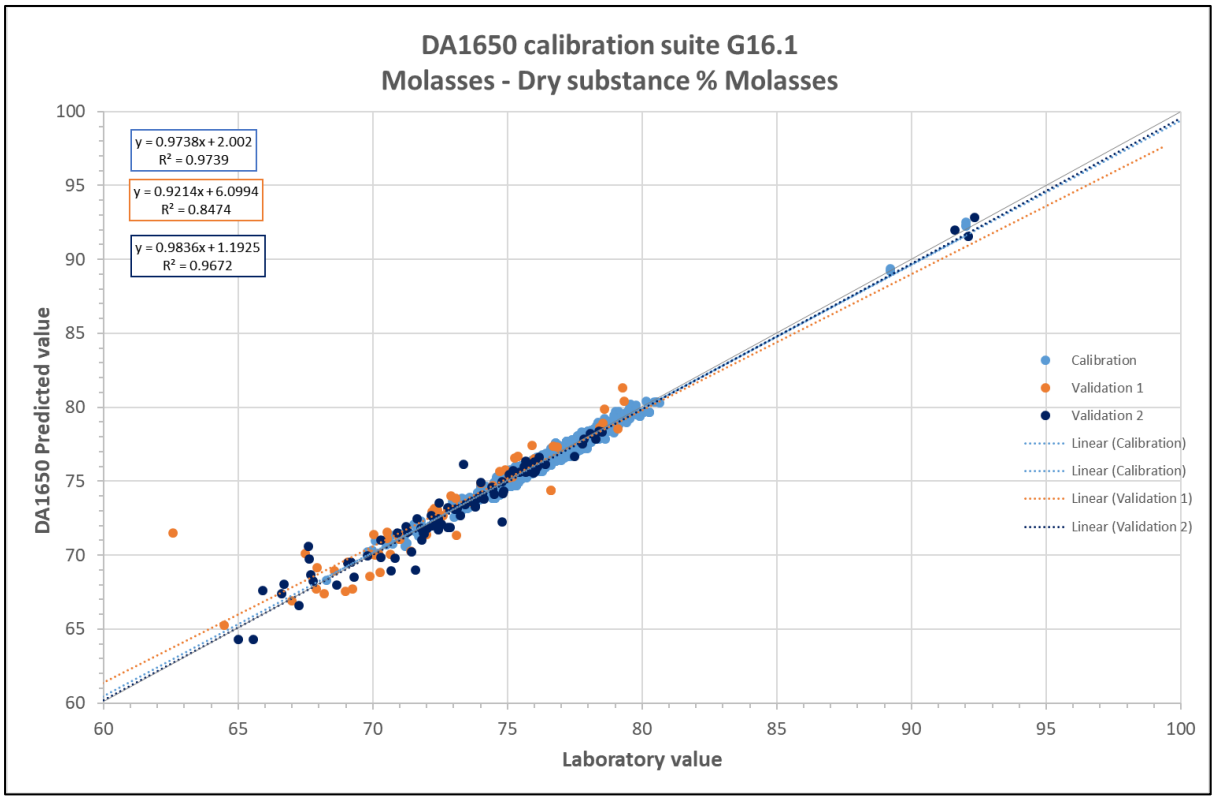

Figure 46: Calibration (blue) and validation (orange and navy) of Global 16.1 molasses dry substance at Mill 2 in 2017

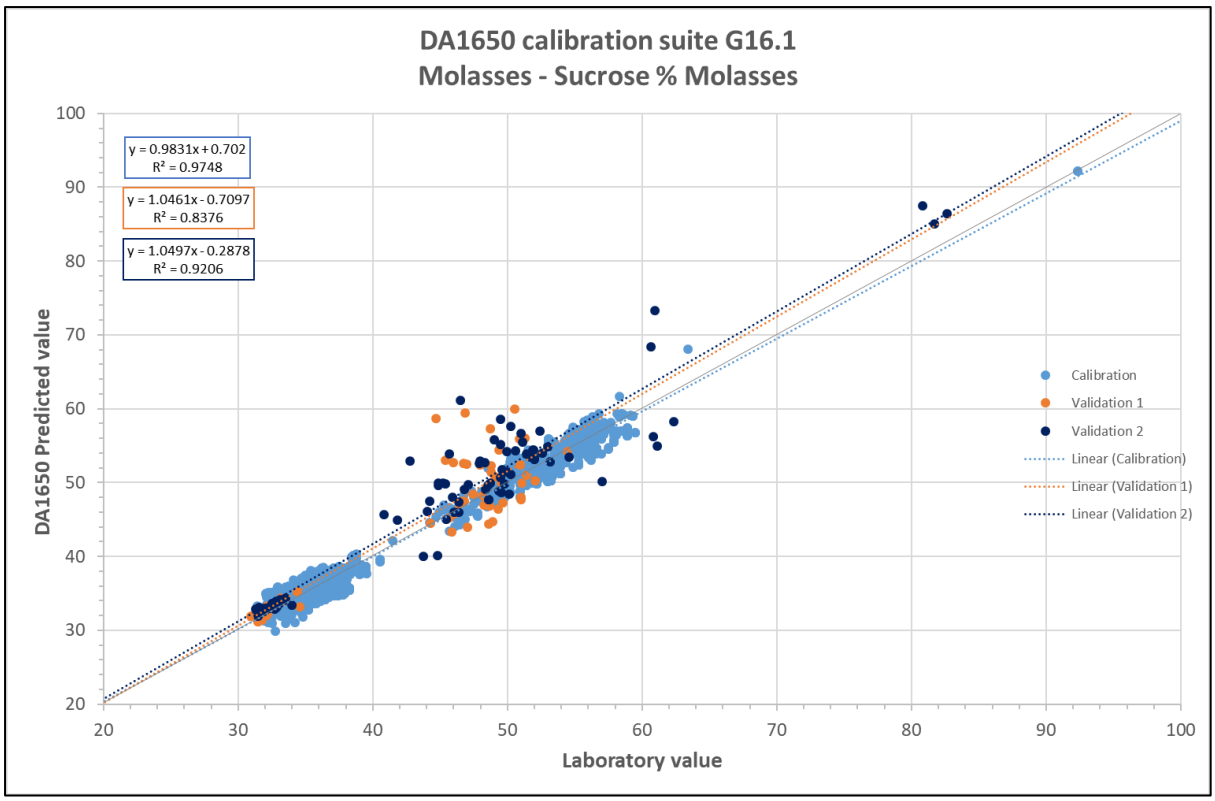

Figure 47: Calibration (blue) and validation (orange and navy) of Global 16.1 molasses sucrose at Mill 2 in 2017

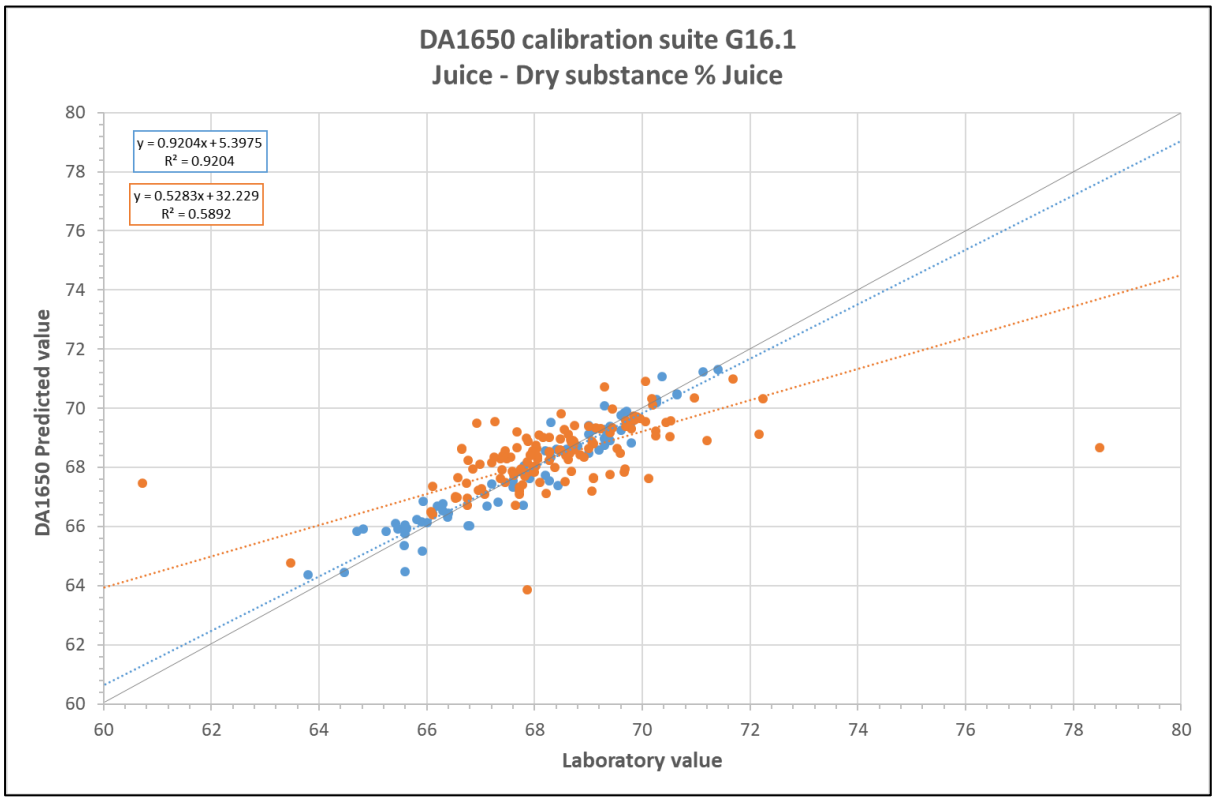

Figure 48: Calibration (blue) and validation (orange) of Global 16.1 juice dry substance at Mill 2 in 2016

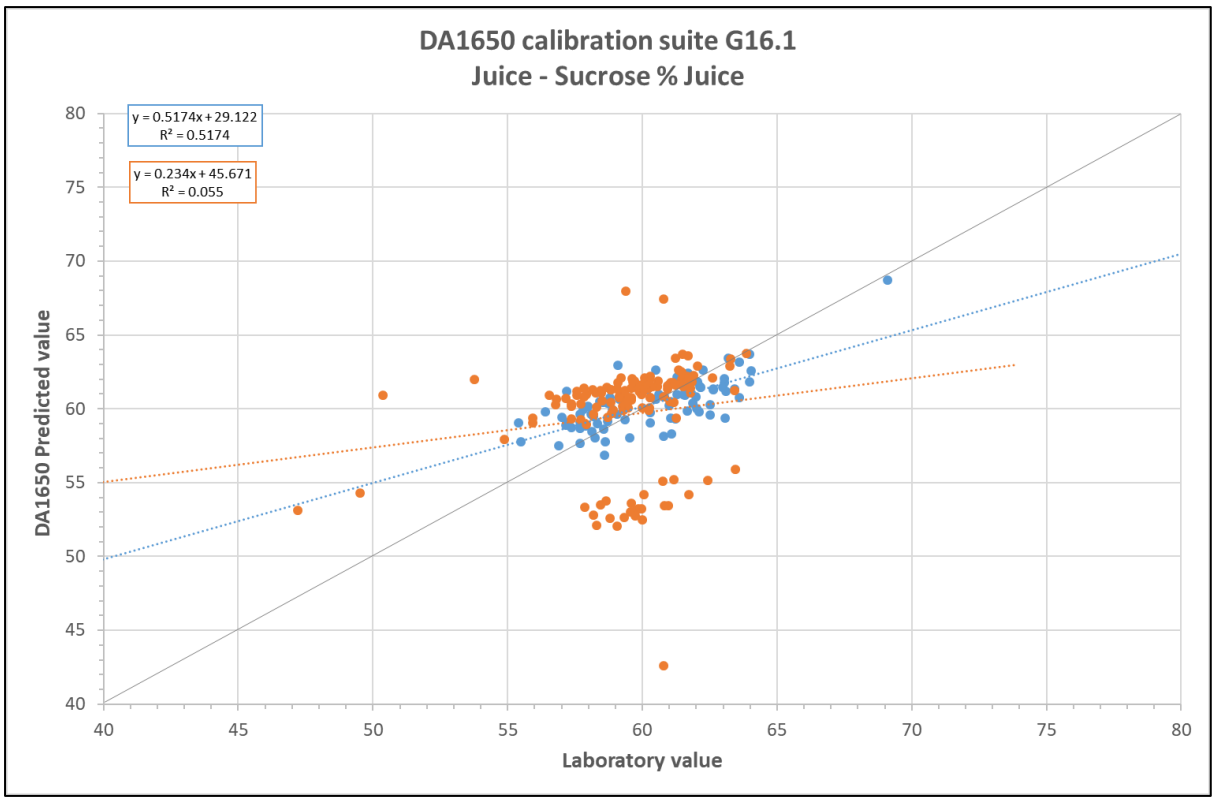

Figure 49: Calibration (blue) and validation (orange) of Global 16.1 juice sucrose at Mill 2 in 2016

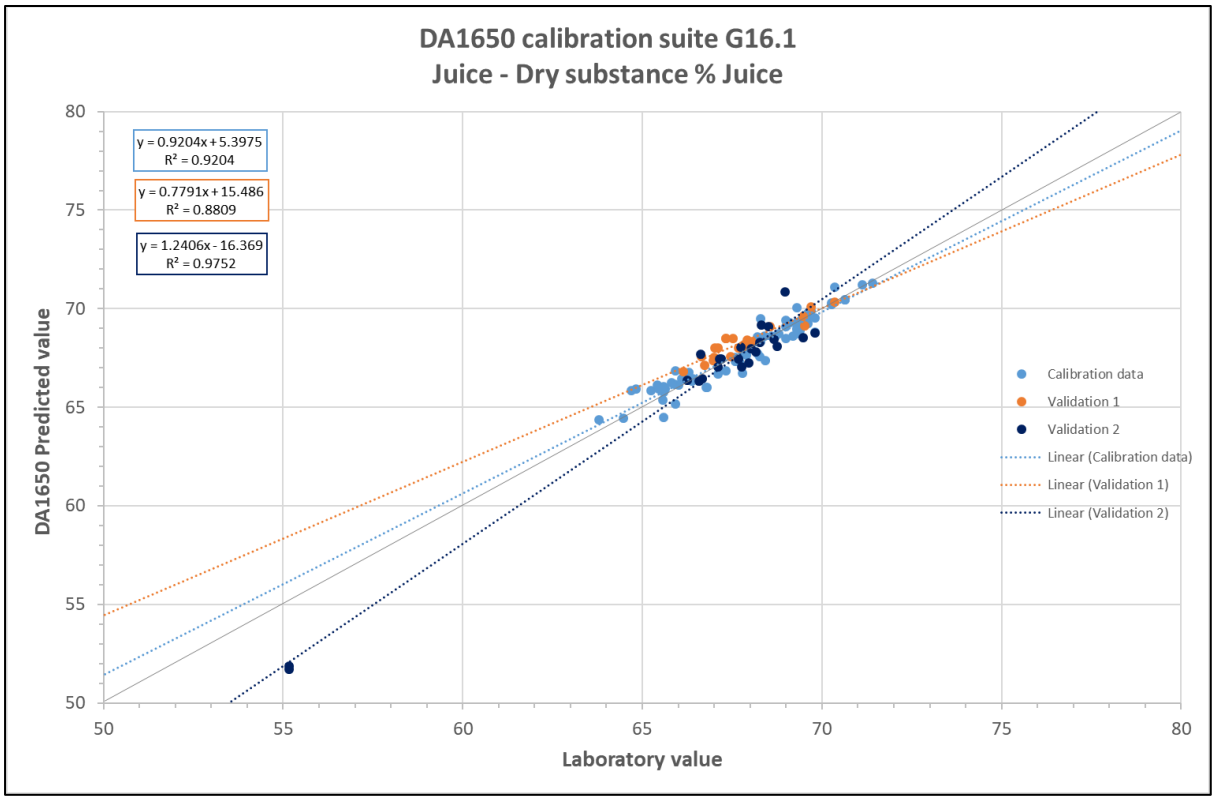

Figure 50: Calibration (blue) and validation (orange and navy) of Global 16.1 juice dry substance at Mill 2 in 2017

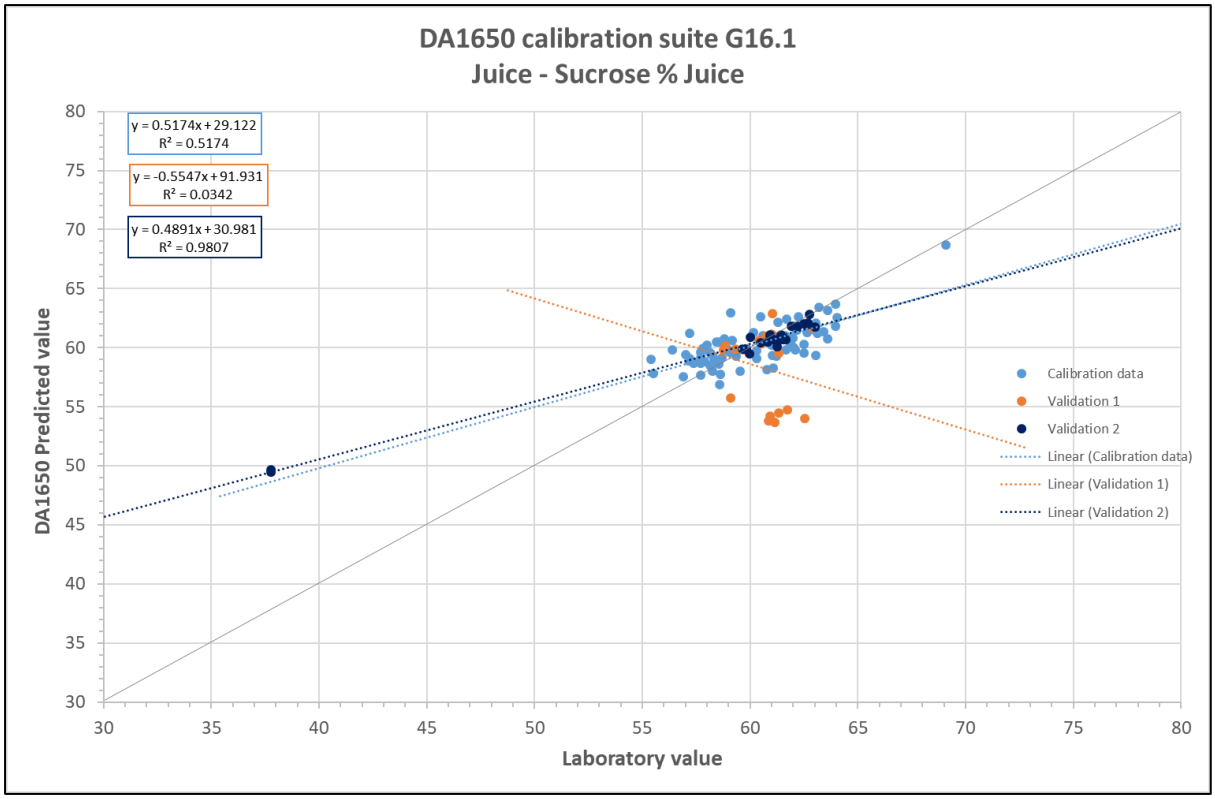

Figure 51: Calibration (blue) and validation (orange and navy) of Global 16.1 juice sucrose at Mill 2 in 2017

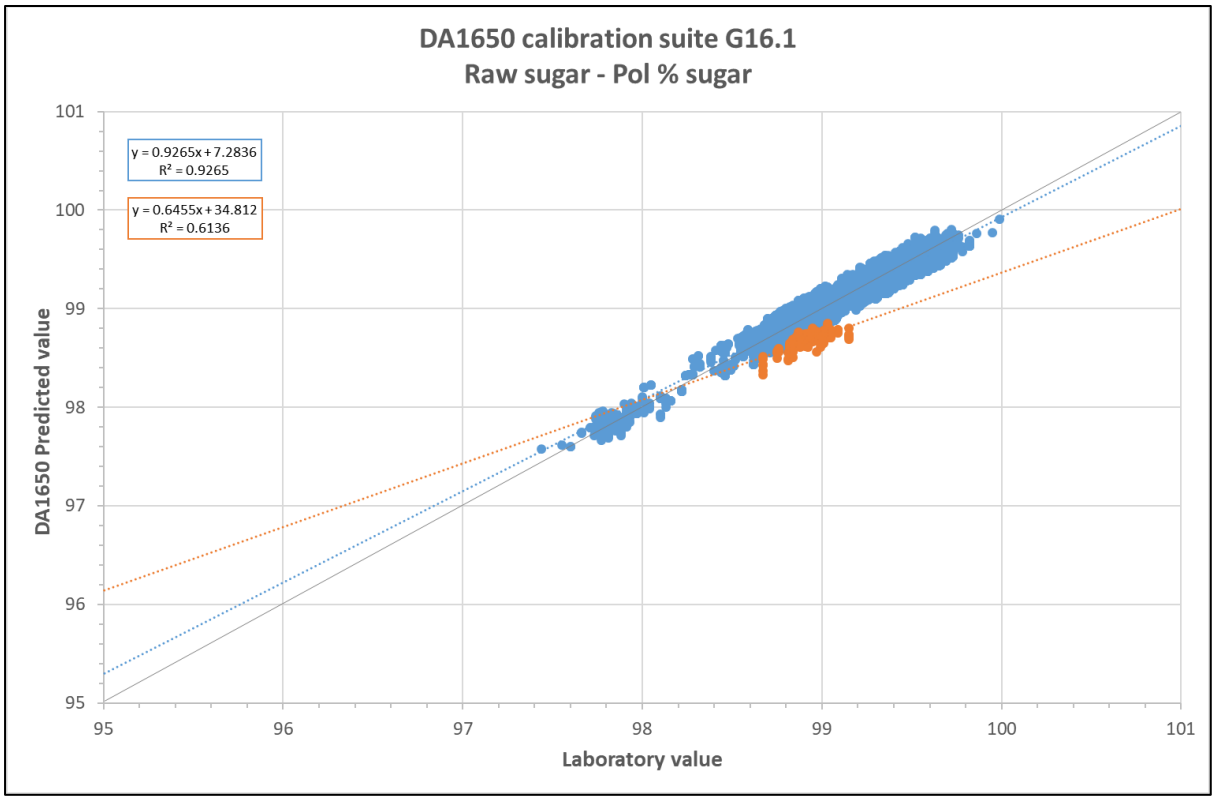

Figure 52: Calibration (blue) and validation (orange) of Global 16.1 raw sugar pol at Mill 1 in 2016

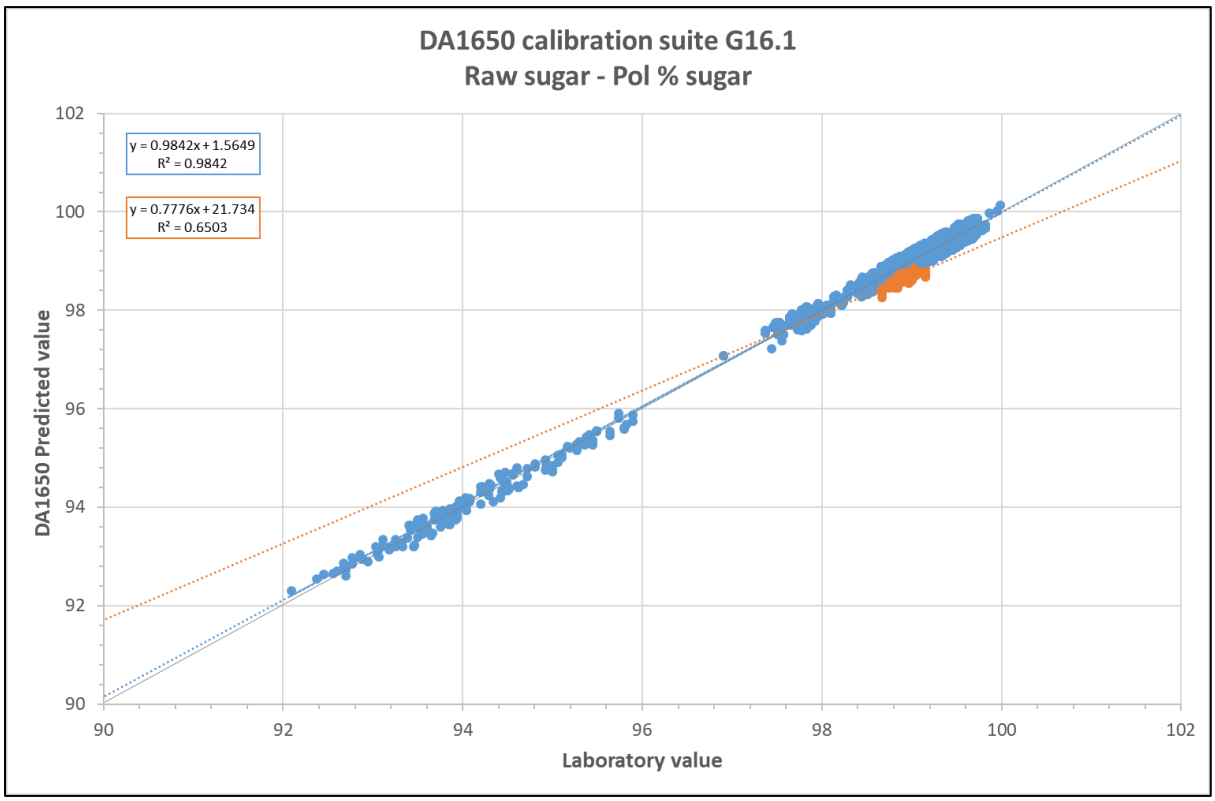

Figure 53: Calibration (blue) and validation (orange) of Global 16.1 raw sugar pol (low purity model) at Mill 1 in 2016

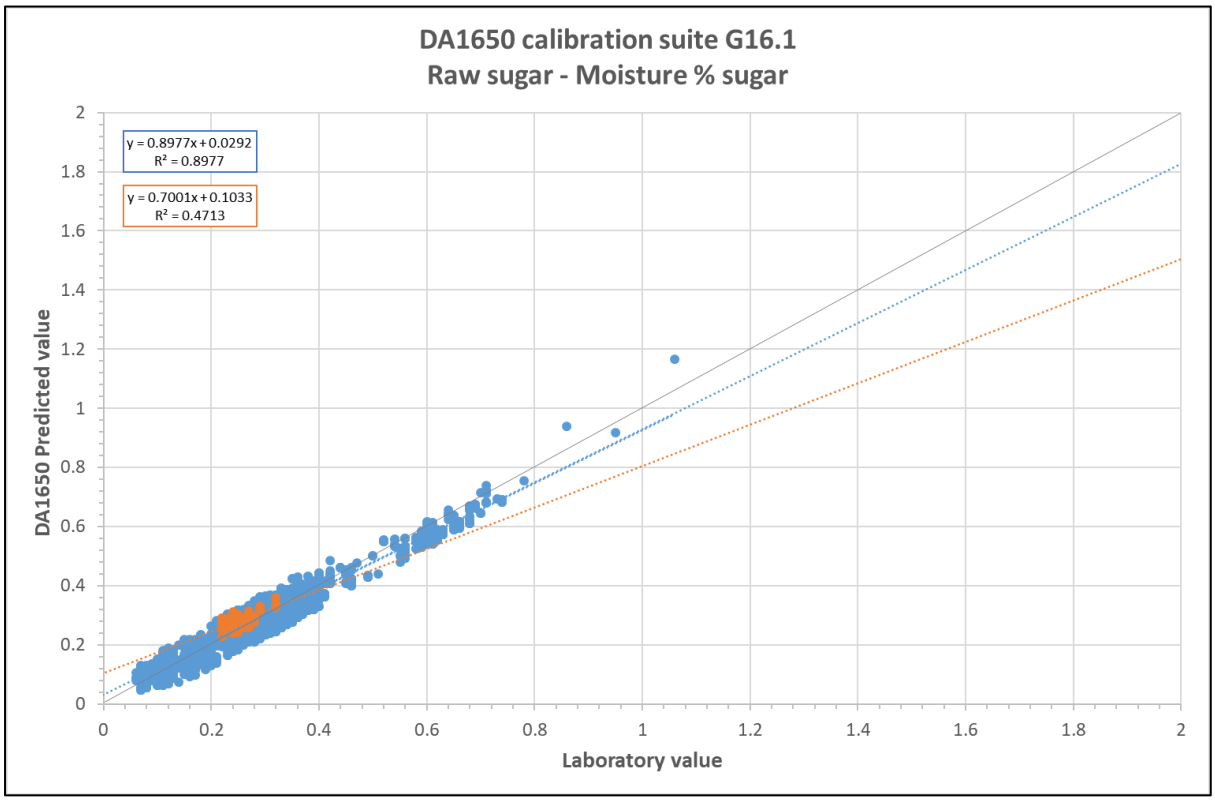

Figure 54: Calibration (blue) and validation (orange) of Global 16.1 raw sugar moisture at Mill 1 in 2016

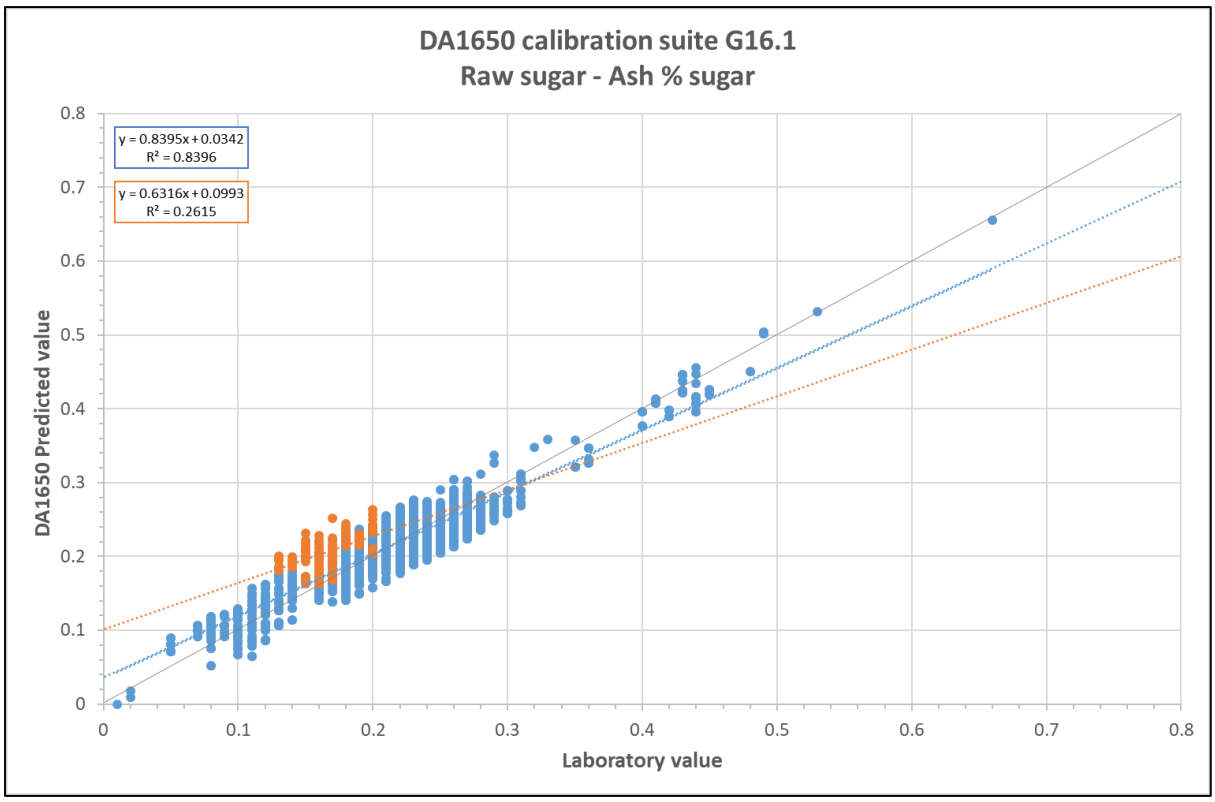

Figure 55: Calibration (blue) and validation (orange) of Global 16.1 raw sugar ash at Mill 1 in 2016

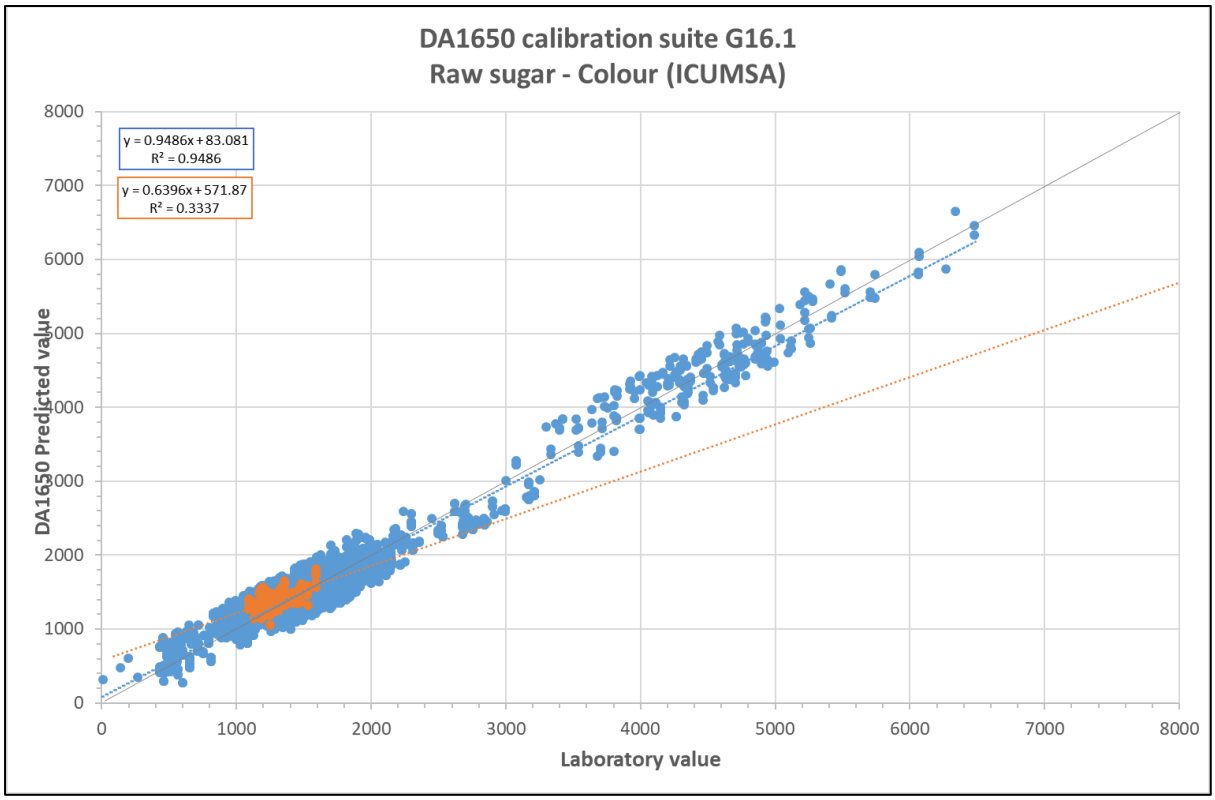

Figure 56: Calibration (blue) and validation (orange) of Global 16.1 raw sugar colour at Mill 1 in 2016

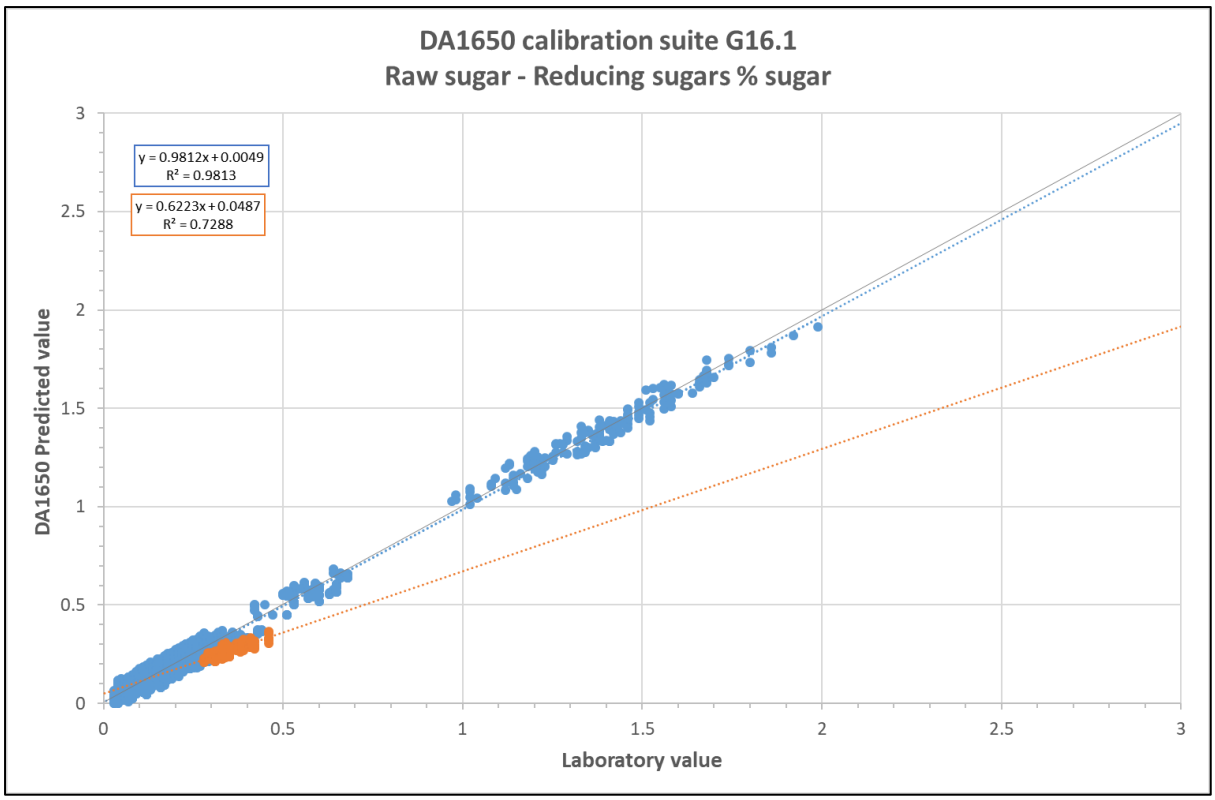

Figure 57: Calibration (blue) and validation (orange) of Global 16.1 raw sugar reducing sugars at Mill 1 in 2016

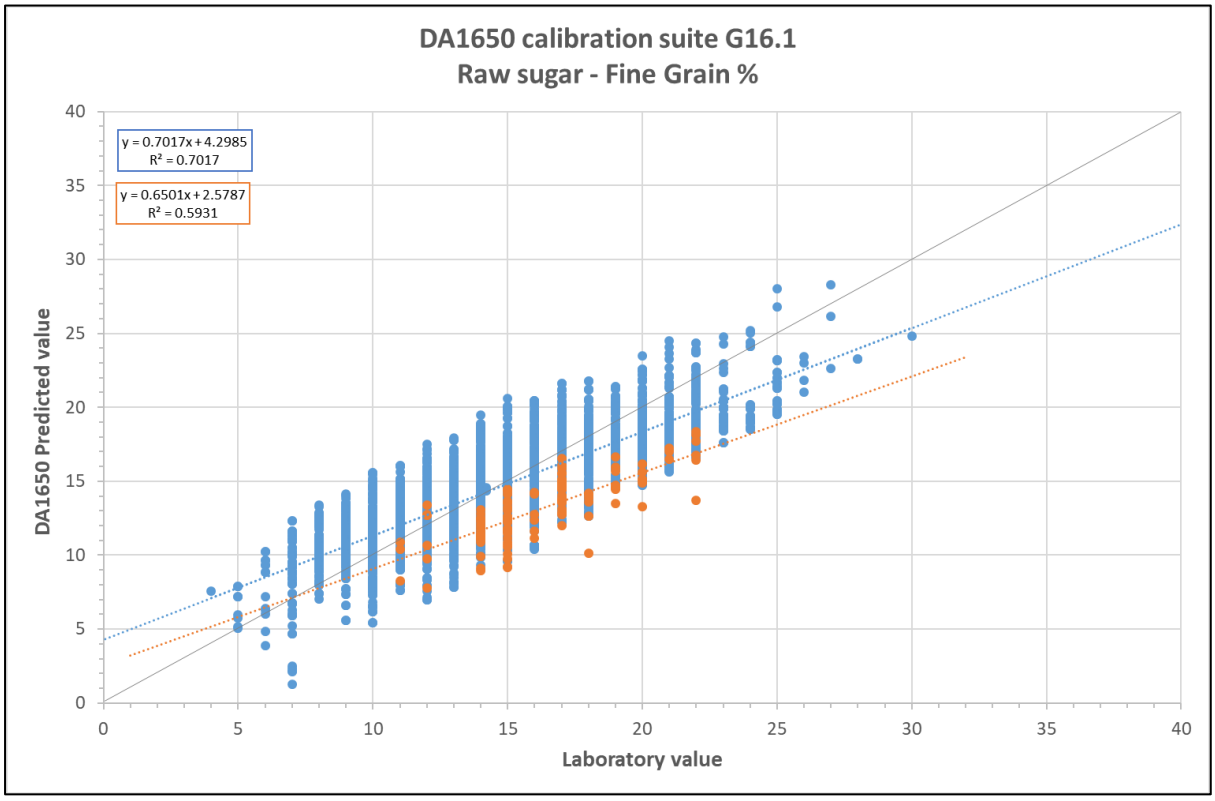

Figure 58: Calibration (blue) and validation (orange) of Global 16.1 raw sugar fine grain at Mill 1 in 2016

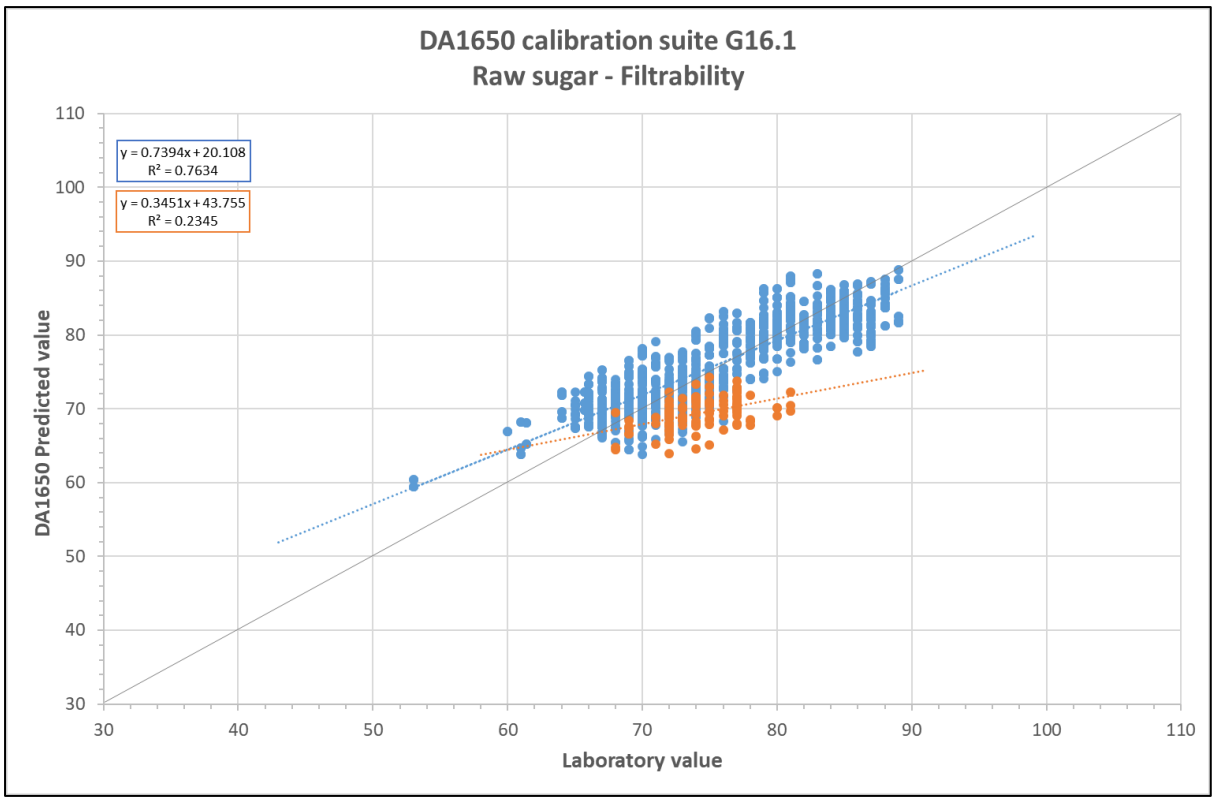

Figure 59: Calibration (blue) and validation (orange) of Global 16.1 raw sugar filtrability at Mill 1 in 2016

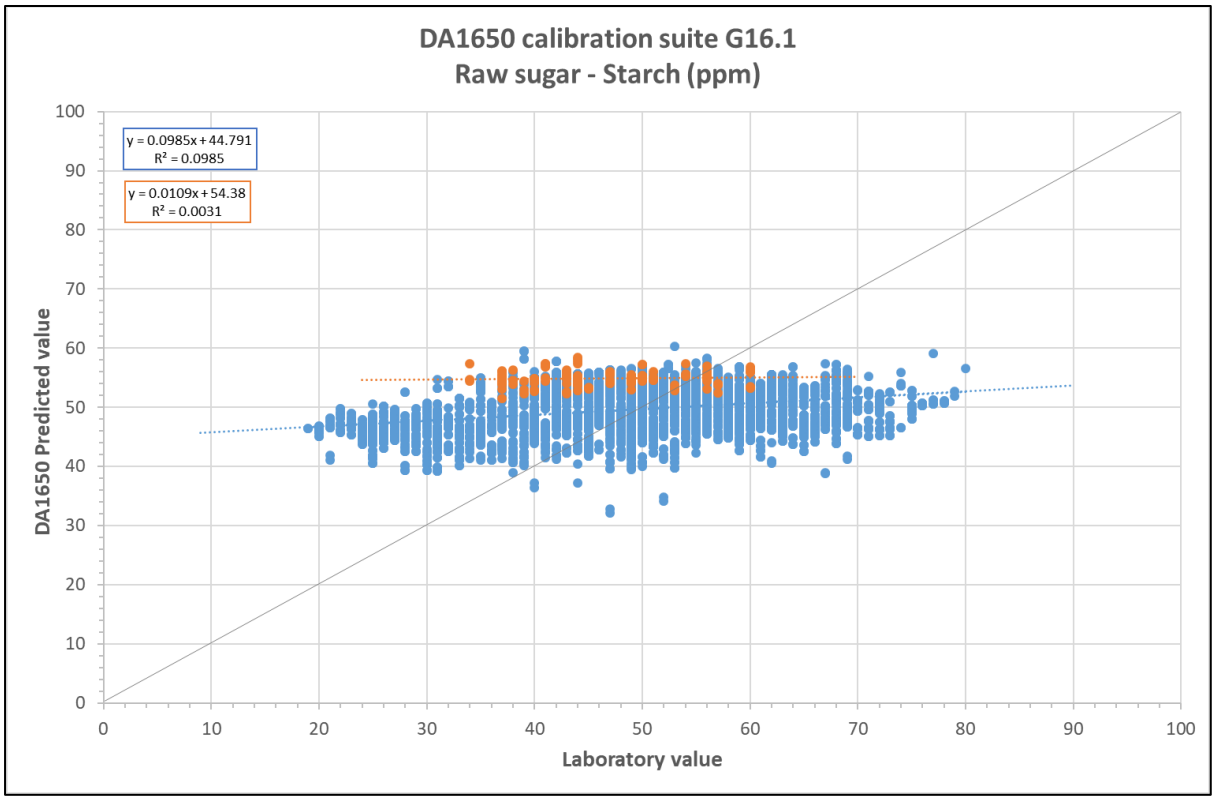

Figure 60: Calibration (blue) and validation (orange) of Global 16.1 raw sugar starch at Mill 1 in 2016

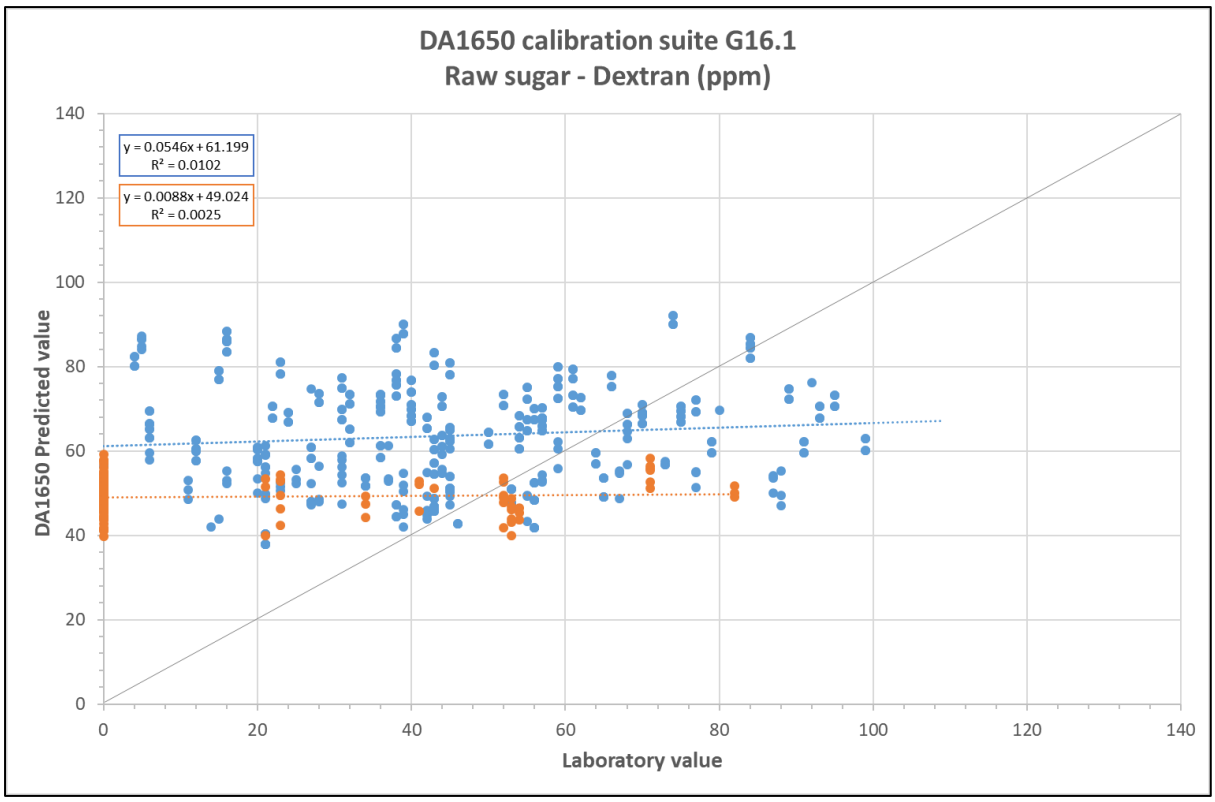

Figure 61: Calibration (blue) and validation (orange) of Global 16.1 raw sugar dextran at Mill 1 in 2016

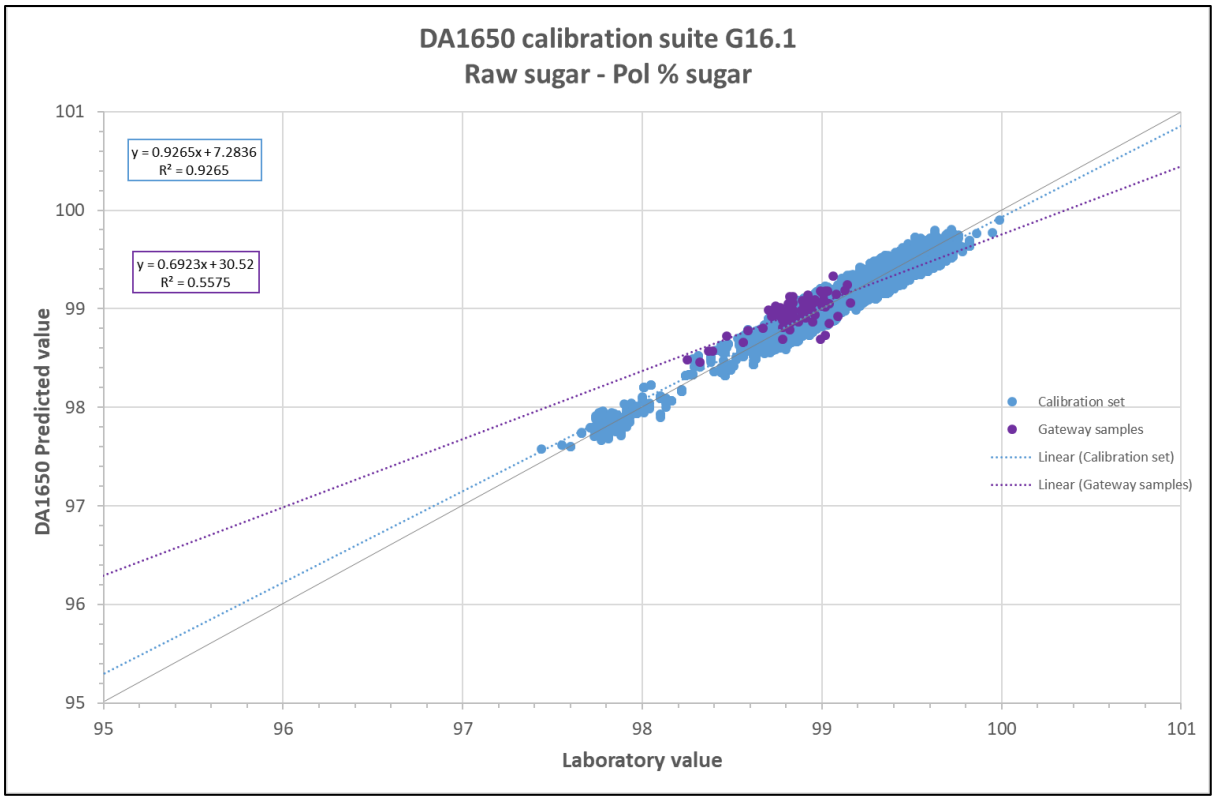

Figure 62: Calibration (blue) and validation (purple) of Global 16.1 raw sugar pol at Mill 2 in 2016

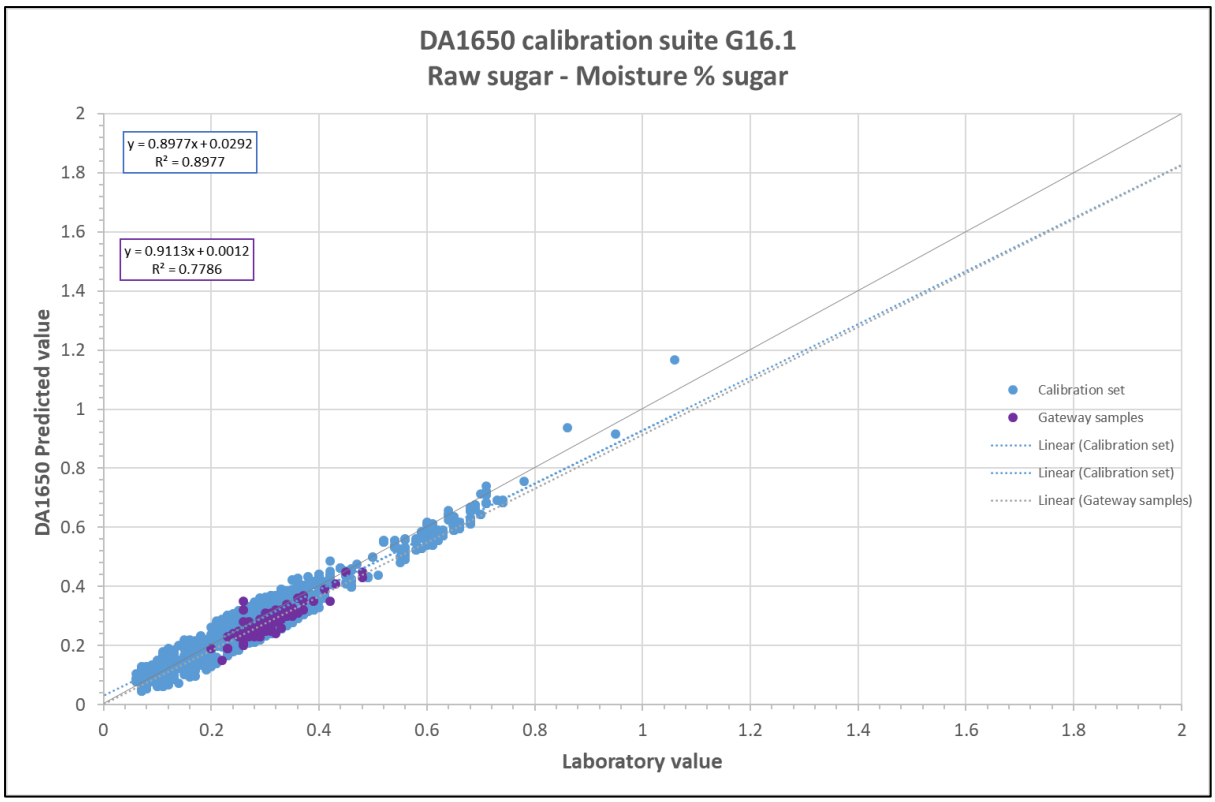

Figure 63: Calibration (blue) and validation (purple) of Global 16.1 raw sugar moisture at Mill 2 in 2016

Due to the performance in 2016, the G16.1 calibration models for raw sugar were not updated in 2017. The validation statistics for Mill 2 and Mill 1 for their 2017 terminal samples are provided in Table 14 and the calibration/validation plots are provided in Figure 64 to Figure 75. Pol (normal and low purity), ash, moisture, colour and reducing sugars all show validation samples that are tightly clustered and sit within the scatter of the calibration samples, although ash shows small bias. This is reflected by the low SEP values, which are all less than or equal to the ECL values. Finegrain also shows a bias and slightly more scatter. Similarly to the 2016 season, starch and dextran did not perform well and it is suggested that calibrations for these constituents are no longer included in the calibration suite.

The errors associated with the raw sugar models are comparable to the SEC values and approaching the laboratory error. The calibration sample size and minimal improvement in the models from the G15.2 models indicates that these models are mature. The SEP values are low enough for the models to be used as operational tools at QSL and Tully Mill.

Table 14: Validation statistics for G16.1 Raw sugar in 2017

	Constituent name	Validation statistics - Mill 2 2017				Validation statistics - Mill 1 2017			
		N	R^2	Bias	SEP	N	R^2	Bias	SEP
Raw sugar	Pol % Sugar	217	0.53	0.03	0.08	129	0.21	0.07	0.06
	Pol - low purity % Sugar					129	0.27	0.07	0.06
	Moisture % Sugar	216	0.55	-0.02	0.03	129	0.23	-0.01	0.02
	Ash % Sugar					129	0.37	0.03	0.02
	Colour (ICUMSA)					129	0.28	26	87
	Reducing Sugars % Sugar					129	0.14	-0.04	0.03
	Fine Grain %					129	0.32	4.78	2.39
	Filtrability					129	0.03	-2.97	3.05
	Starch (ppm)					129	0.01	2.30	8.40
	Dextran (ppm)					129	0.08	-52.46	17.02

N: number of samples, R^2: coefficient of determination, SEP: standard error of prediction

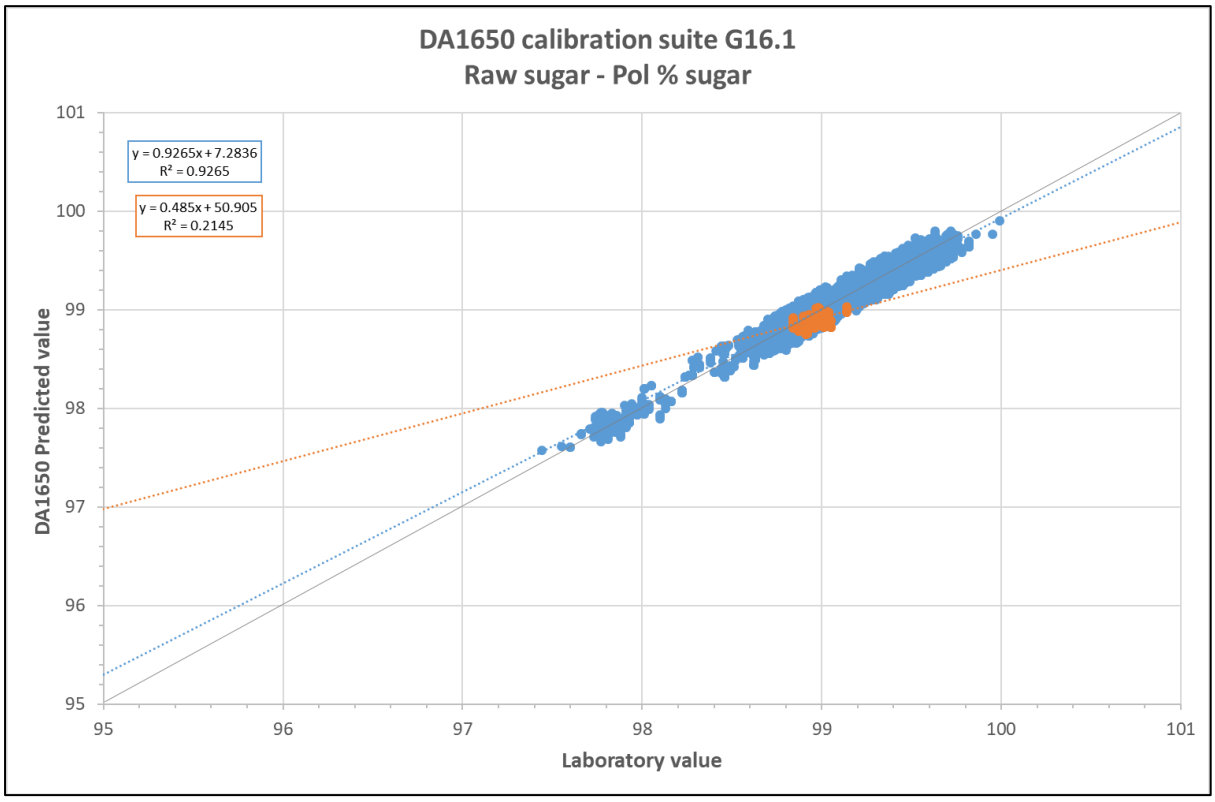

Figure 64: Calibration (blue) and validation (orange) of Global 16.1 raw sugar pol at Mill 1 in 2017

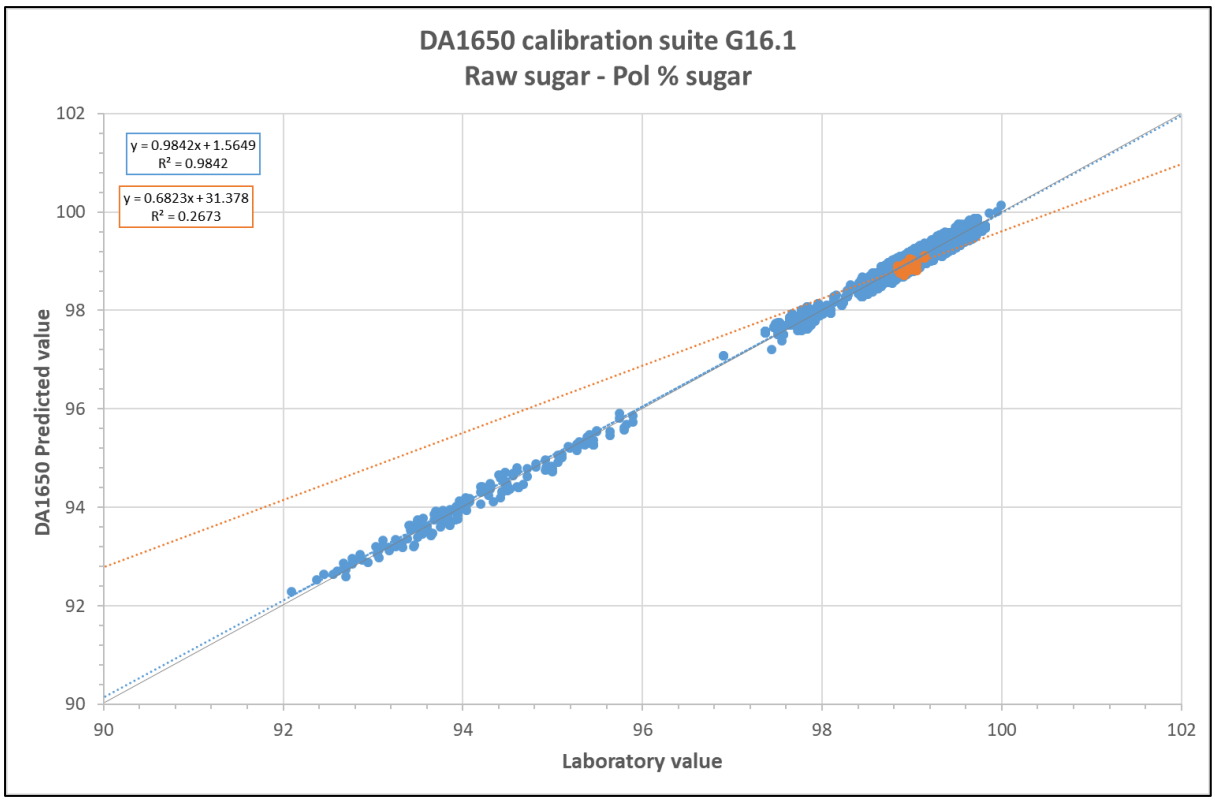

Figure 65: Calibration (blue) and validation (orange) of Global 16.1 raw sugar pol (low purity) at Mill 1 in 2017

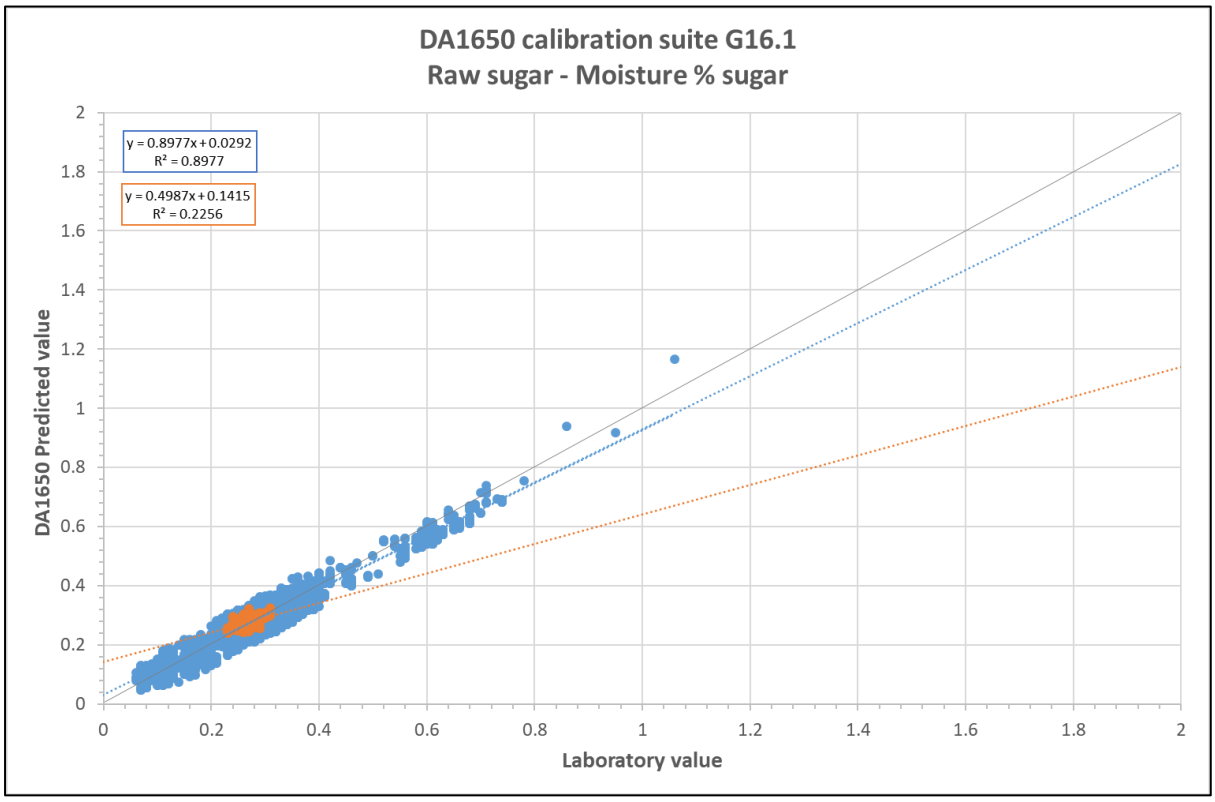

Figure 66: Calibration (blue) and validation (orange) of Global 16.1 raw sugar moisture at Mill 1 in 2017

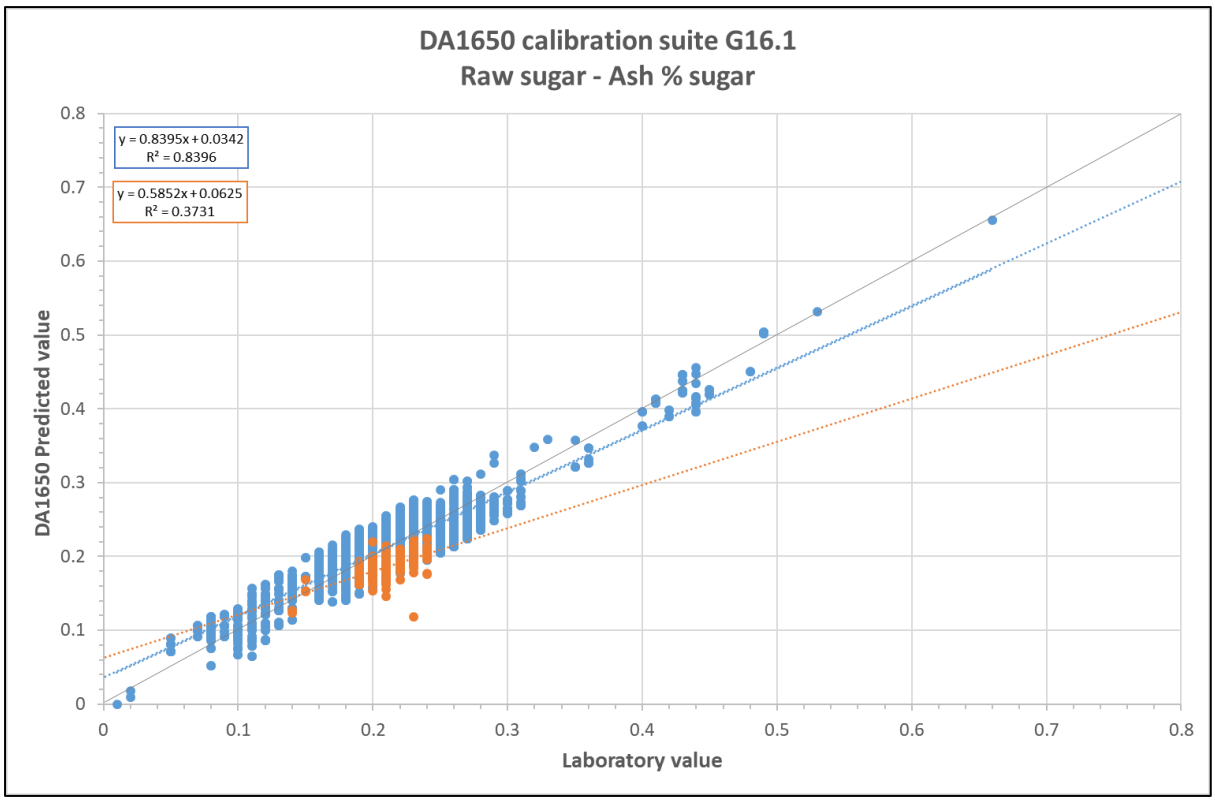

Figure 67: Calibration (blue) and validation (orange) of Global 16.1 raw sugar ash at Mill 1 in 2017

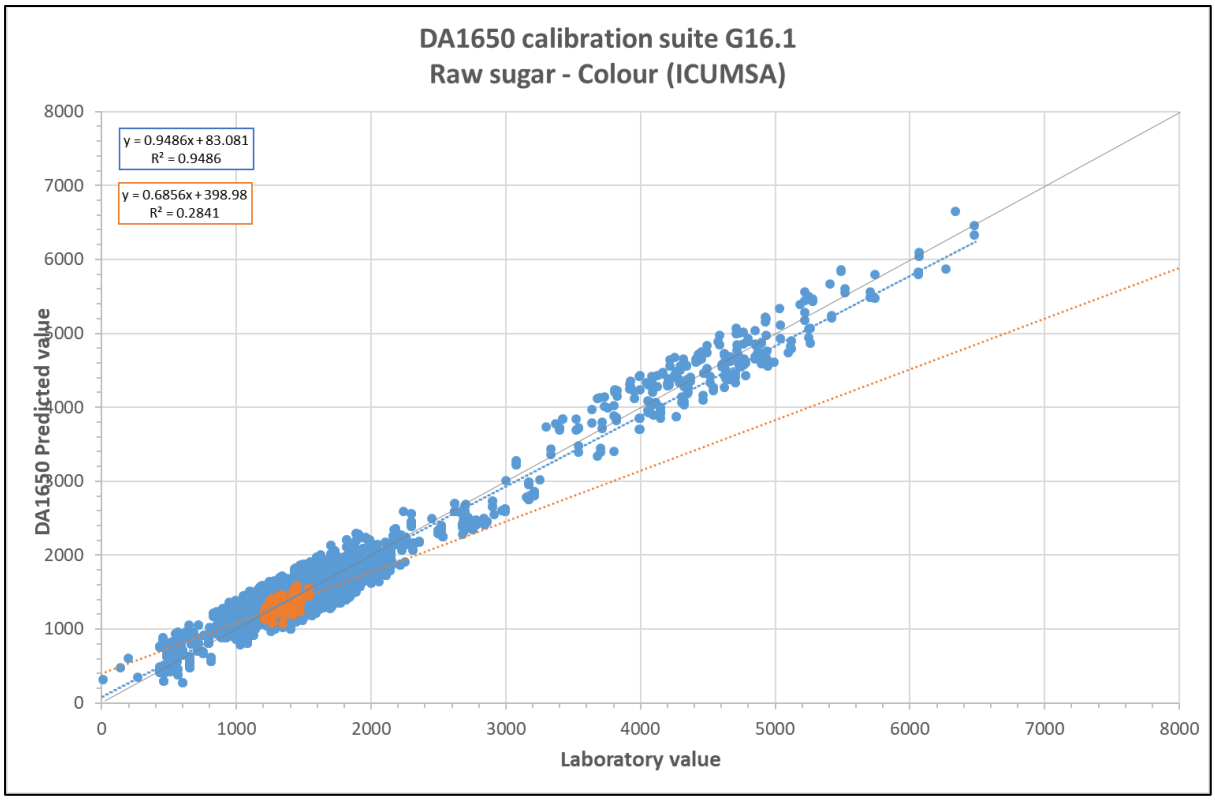

Figure 68: Calibration (blue) and validation (orange) of Global 16.1 raw sugar colour at Mill 1 in 2017

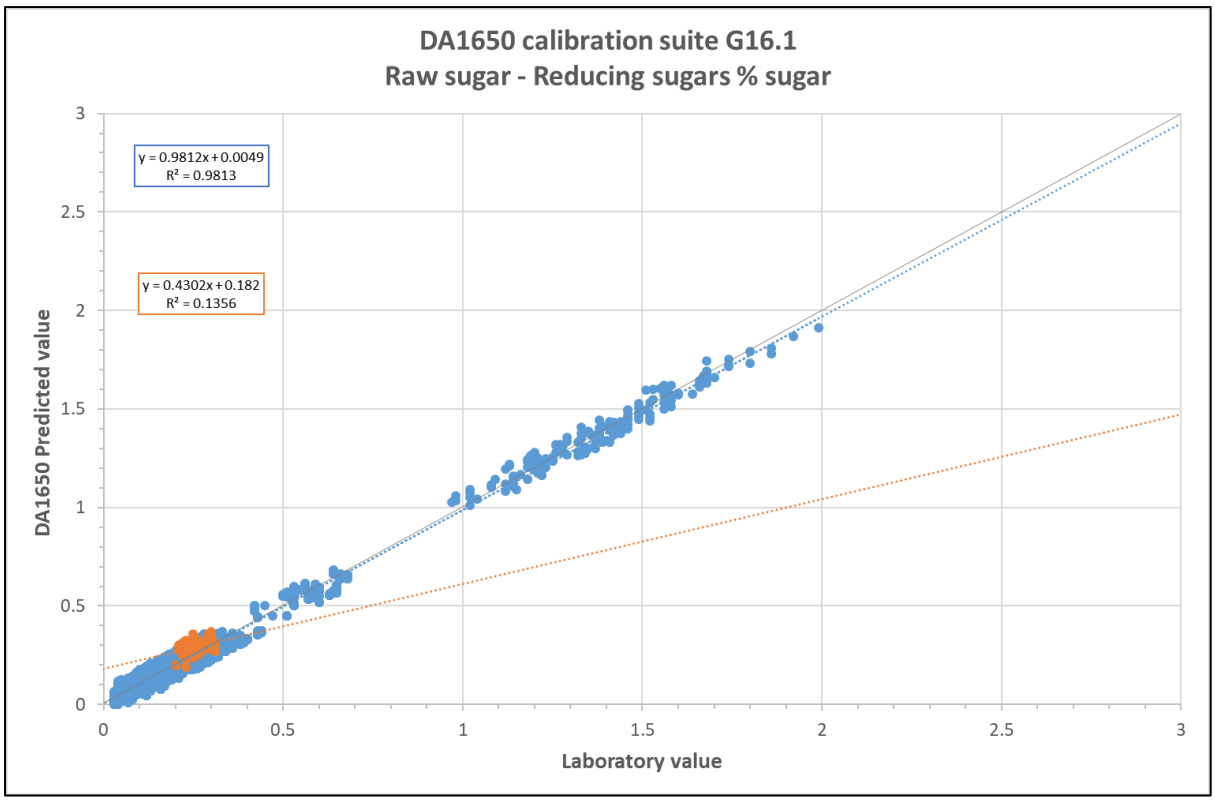

Figure 69: Calibration (blue) and validation (orange) of Global 16.1 raw sugar reducing sugars at Mill 1 in 2017

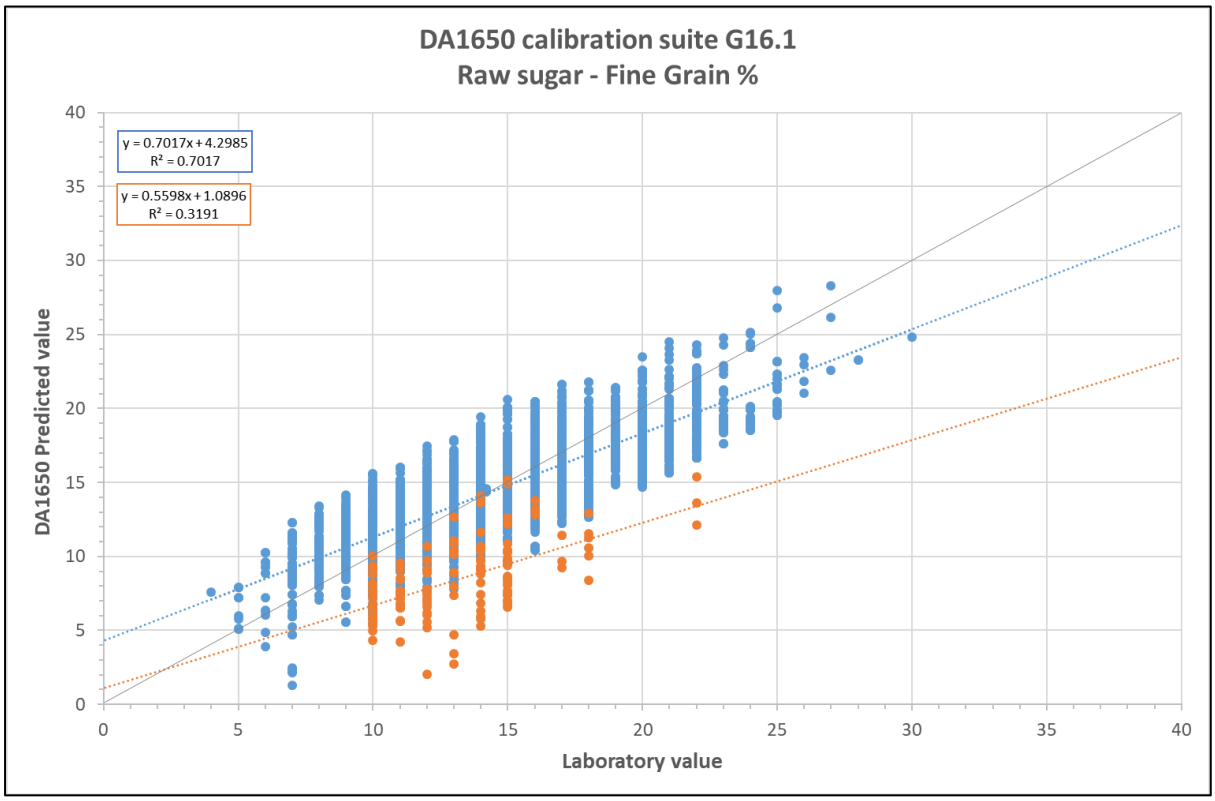

Figure 70: Calibration (blue) and validation (orange) of Global 16.1 raw sugar fine grain at Mill 1 in 2017

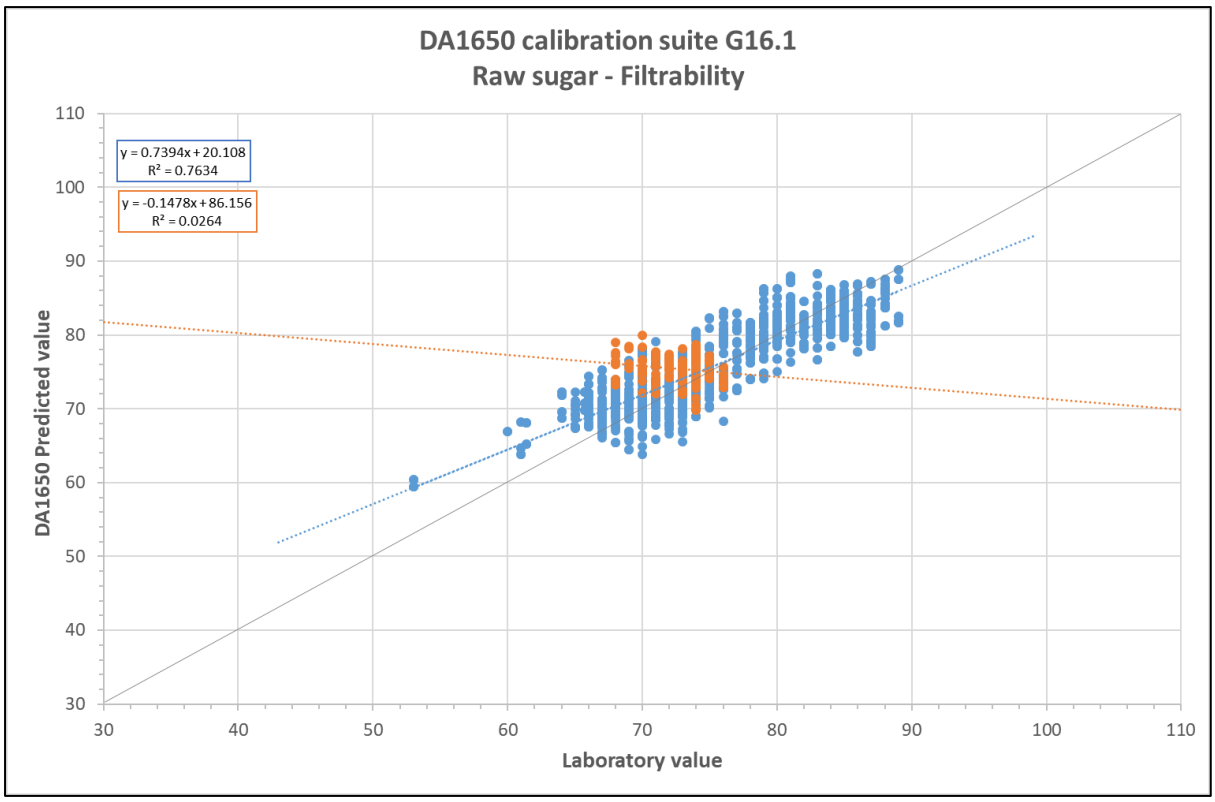

Figure 71: Calibration (blue) and validation (orange) of Global 16.1 raw sugar filtrability at Mill 1 in 2017

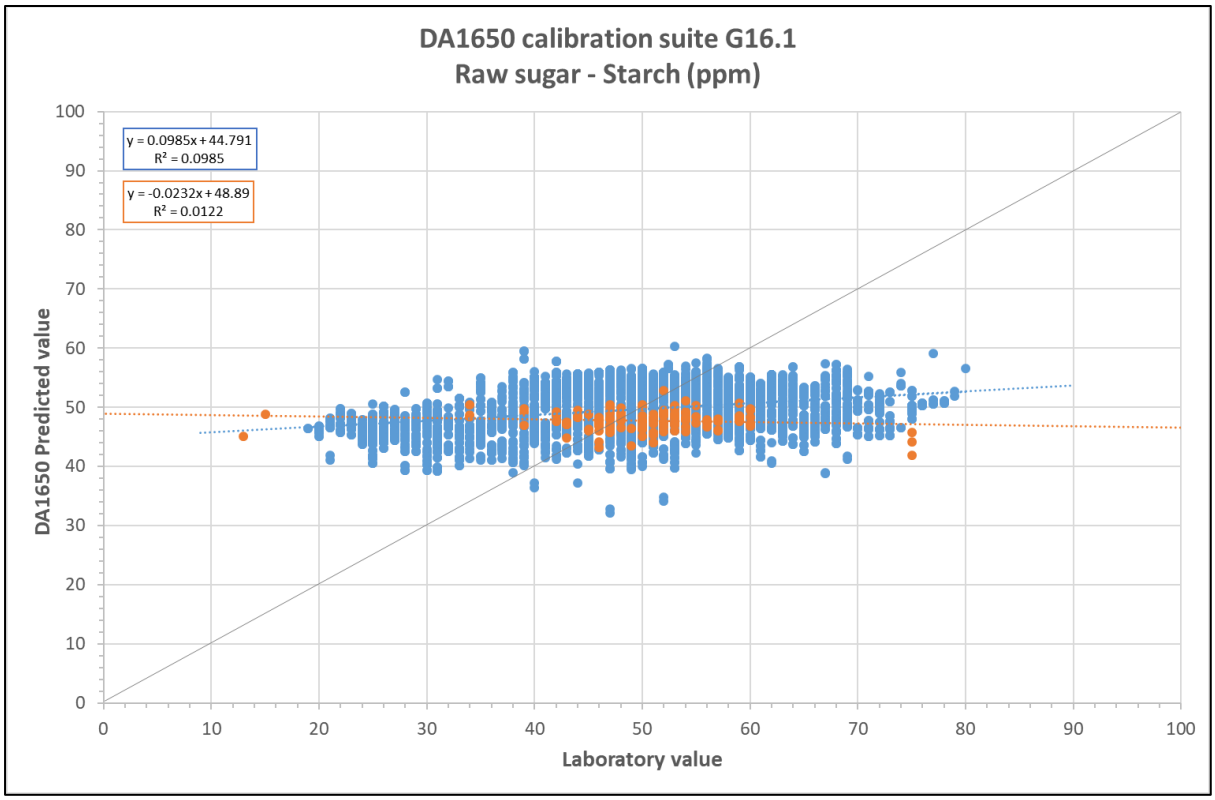

Figure 72: Calibration (blue) and validation (orange) of Global 16.1 raw sugar starch at Mill 1 in 2017

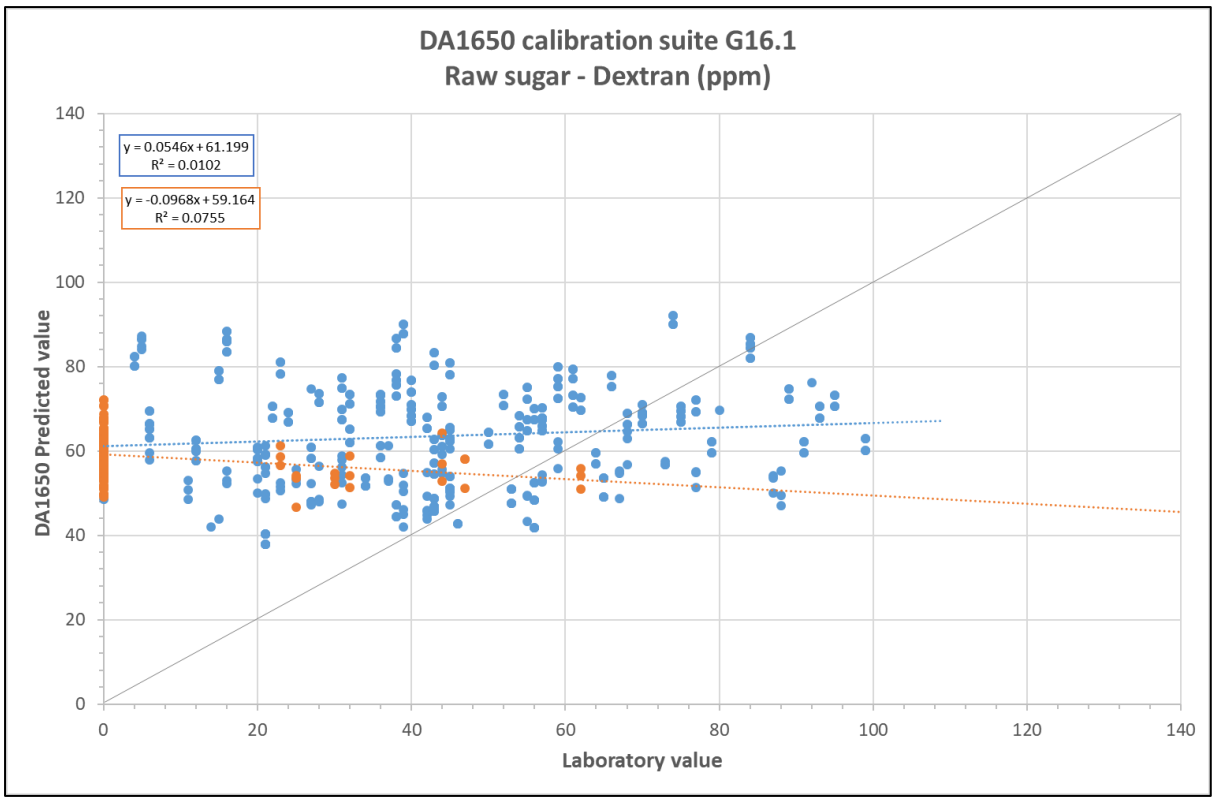

Figure 73: Calibration (blue) and validation (orange) of Global 16.1 raw sugar dextran at Mill 1 in 2017

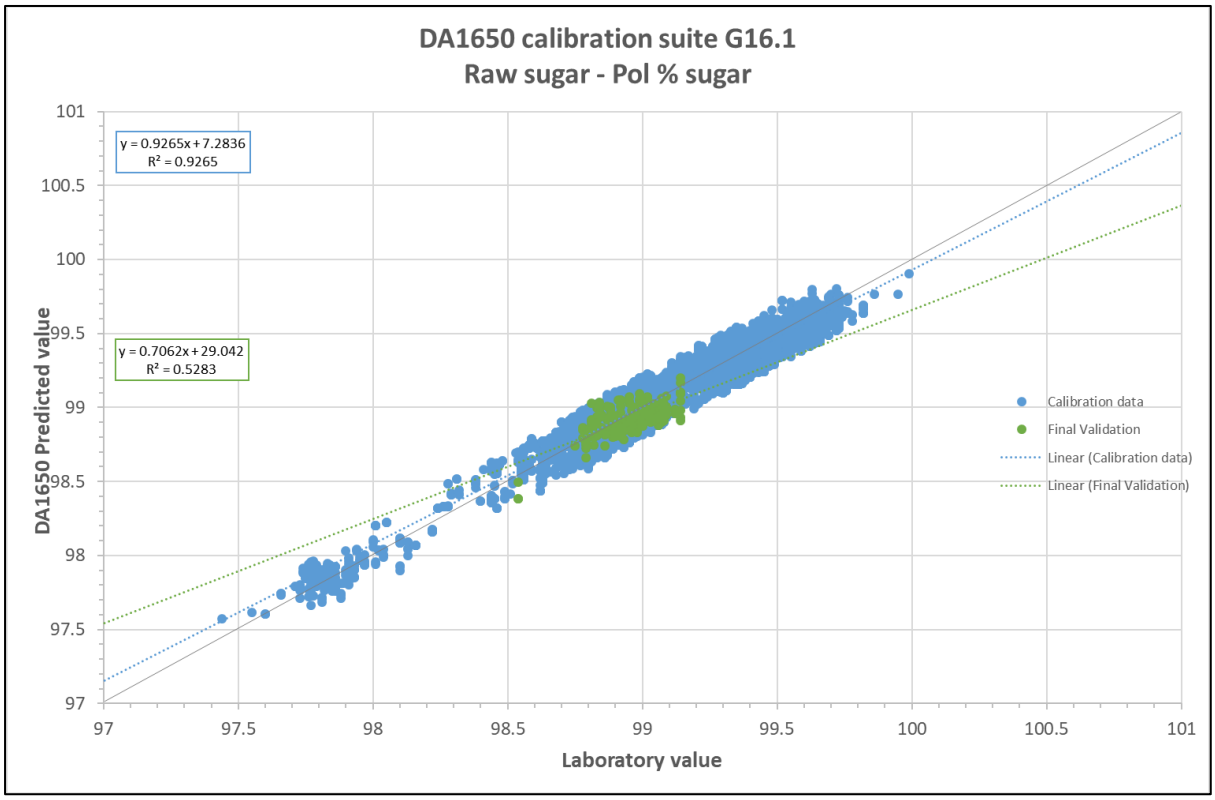

Figure 74: Calibration (blue) and validation (green) of Global 16.1 raw sugar pol at Mill 2 in 2017

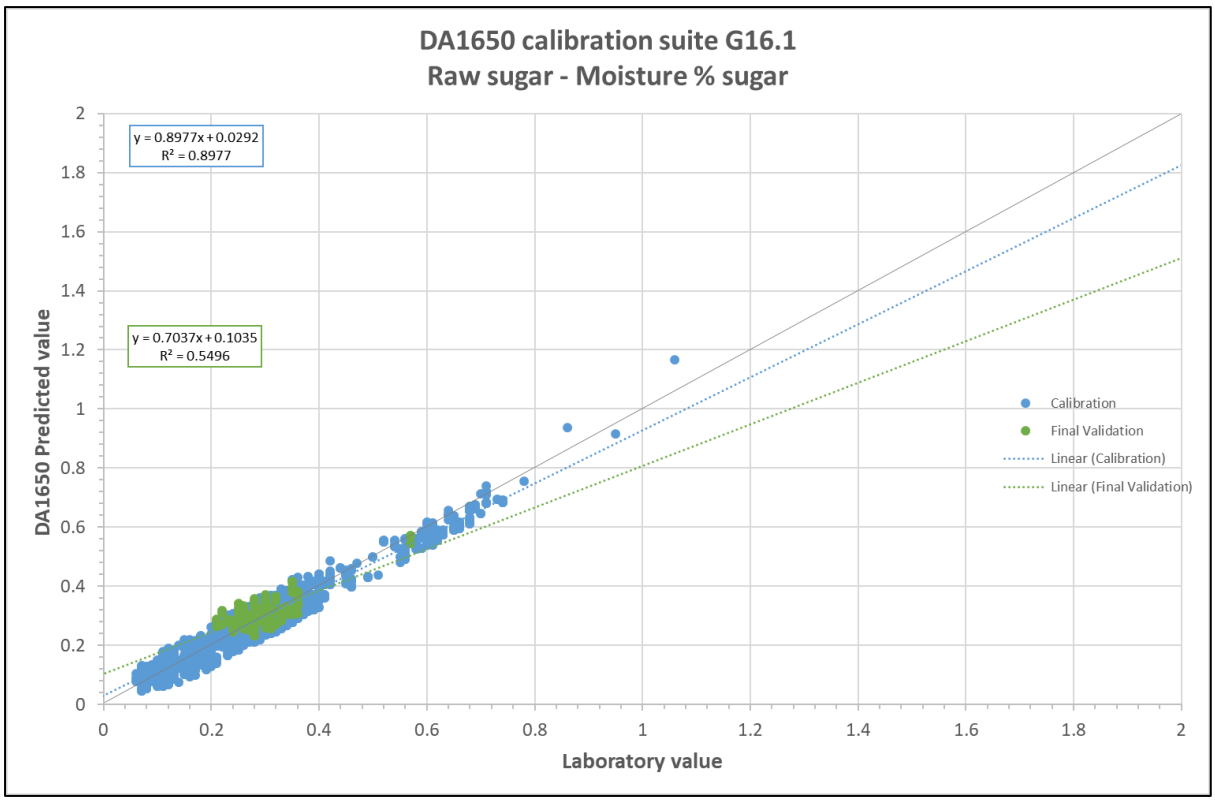

Figure 75: Calibration (blue) and validation (green) of Global 16.1 raw sugar moisture at Mill 2 in 2017

6.1.3.5. Global 17.1

After several weeks of operation with the G16.1 models in the 2017 season, it was apparent that the pan product models were not improving on the previous years' performance (Section 6.1.3.5). Re-calibration was required to improve the prediction of the erroneous samples. For each product, several iterations were attempted to improve the models recognition and predictive ability for the samples and included splitting sub products (e.g. A and B molasses and C molasses), using DA1650 data only, cleaning the spectral dataset and changing the structure of the spectral data. Ultimately, the best performance was achieved by using specialised, in-house software to remove spectra affected by significant levels of noise (which included a large amount of old XDS and InfraXact™ data), changing the spectral data structure to a 2 nm data frequency between 1100 and 1650 nm, and forcing all calibration models to 16 factors. The data-cleaning step reduced the number of samples available for calibration, particularly for molasses, but provided a more specific set for the current application. Molasses samples tend towards higher levels of noise as they are highly absorbing and force the detector to operate at its bounds (Section 6.3.2.3).

New calibration models were developed for the dry substance and sucrose constituents of molasses, massecuite and juice models, as they were the products with new data available. The remaining constituents in the G17.1 calibration suite were carried over from G16.1. True purity models were also developed for each pan product. The calibration and validation statistics for the G17.1 models are provided in Table 15 and the associated calibration/validation plots are provided in Figure 76 to Figure 84.

Overall, the re-calibration provided better predictive performance for each of the constituents, represented by an improvement in SEP from the G16.1 models. This is despite slight increases in the SEC values for each model. This indicates that there has been a reduction in the specificity of the model, allowing inclusion of the samples with the unknown composition causing the scatter.

Although the scatter has improved for most models, some variation can be seen, although it is different for the two mills. Mill 1 shows more variability in the massecuite models, particularly for dry substance, but shows very little scatter in the molasses and juice models. Mill 2 is the opposite. The presence of the scatter suggests that all of the sample variability has not yet been captured and the models will continue to need updating for the near future. For both mills, the predictions for C molasses, which is one of the key constituents of interest to minimise losses in the pan stage, are very high quality. The SEP value for Mill 1 sucrose in C molasses only is 0.71 and the SEP value of Mill 2 sucrose in C molasses only is 0.67.

The juice models still show a large amount of scatter. These models have less than 500 samples and are unlikely to fully represent the sample variation, particularly with regard to the unknown cause of scatter. Continuing to expand the dataset and re-calibrate will likely improve this model to provide acceptable performance. It is also possible that the sample presentation is causing some issues for this model. The juice and syrup model uses a gold reflector to provide a 0.4 cm transflectance cell, designed for transparent materials. If care is not taken when using this accessory, bubbles in the read area can give erroneous results.

Table 15: Calibration and validation statistics for G17.1 calibration models

	Constituent name	Calibration statistics									Validation - Mill 2				Validation - Mill 1			
		SEC	R^2	Mean	SD	Min	Max	N	ECL	Facs.	N	R^2	Bias	SEP	N	R^2	Bias	SEP
Molasses	Dry Substance % Molasses	0.51	0.96	75.92	2.55	64.29	82.87	1169	0.62	16	337	0.97	0.02	0.56	118	0.79	0.35	0.79
	Ash % Molasses	0.30	0.82	13.40	0.71	11.18	15.34	364	0.36	14								
	Reducing sugars % Molasses	0.86	0.88	12.21	2.50	8.16	17.31	513	1.03	13								
	Sucrose % Molasses	1.18	0.98	44.52	8.53	31.30	58.34	1211	1.42	16	343	0.97	0.09	1.39	116	0.99	0.60	1.45
	Brix % Molasses	0.94	0.98	82.51	7.55	12.40	99.40	1799	1.13	8								
	Pol % Molasses	1.44	0.98	44.53	10.65	26.37	81.14	1577	1.72	8								
	True purity	1.72	0.98	58.34	11.85	40.28	75.78	1209	2.06	16	343	0.97	-0.52	2.06	116	0.99	0.42	1.75
	Acetic acid % Molasses	0.81	0.89	99.40	2.44	3.22	19.60	53	0.97	8								
	Lactic acid % Molasses	1.48	0.96	81.14	7.03	7.94	39.29	54	1.78	8								
Massecuite and magma	Brix % Massecuite	0.64	0.99	93.18	5.98	68.20	101.7	1574	0.77	14								
	Pol % Massecuite	1.21	0.99	67.35	14.72	31.90	87.17	1580	1.45	16								
	Dry Substance % Massecuite	0.62	0.97	87.53	4.17	72.62	94.70	2819	0.75	16	117	0.39	0.09	0.73	139	0.83	0.08	0.40
	Sucrose % Massecuite	1.15	0.95	73.57	5.79	55.51	88.56	3132	1.38	16	117	0.70	0.61	0.87	139	0.23	0.52	2.34
	Ash % Massecuite	0.16	0.95	13.68	0.70	12.05	15.34	134	0.19	10								
	Reducing Sugars % Mass.	0.27	0.94	9.95	1.10	8.16	13.01	141	0.32	10								
	Crystal % Massecuite	2.55	0.86	43.12	6.90	30.00	55.60	68	3.05	6								
	Massecuite Impurity:water	0.10	0.98	0.92	0.71	0.26	4.56	1189	0.12	12								
	Water % Massecuite	0.53	0.99	14.08	4.69	5.30	27.96	1017	0.64	10								
	True purity	1.61	0.96	83.86	8.20	57.02	95.86	3825	1.93	16	117	0.55	0.56	1.07	139	0.81	0.77	1.79
Juice and syrup	Brix % Juice	0.73	1.00	43.75	28.38	0.10	78.60	10975	0.88	14								
	Pol % Juice	0.63	1.00	30.99	24.96	0.00	73.20	6764	0.75	16								
	Pol Reading (°Z)	0.67	1.00	34.31	27.20	2.04	94.82	5666	0.81	9								
	Ash % Juice	0.24	0.99	5.03	2.85	0.12	11.88	1688	0.29	12								
	Reducing Sugars % Juice	0.07	0.92	0.19	0.23	0.02	3.40	1740	0.08	11								
	CCS	1.01	0.71	13.15	1.88	9.77	16.36	29	1.21	3								
	Sucrose % Juice	0.58	0.96	59.96	3.10	37.78	63.86	491	0.70	16	113	0.16	1.07	1.78	30	0.62	0.99	0.86
	Dry substance % Juice	0.53	0.93	67.90	2.00	55.16	72.24	497	0.64	16	113	0.07	0.49	2.10	30	0.79	0.61	0.63
	True purity	1.51	0.63	88.59	2.47	77.29	95.05	1797	1.81	16	113	0.02	5.54	17.60	30	0.03	0.85	1.29

SD: standard deviation, N: number of samples, R^2: coefficient of determination, SEC: standard error of calibration, ECL: error control limit, Facs.: number of factors, SEP: standard error of prediction, CCS: commercial cane sugar

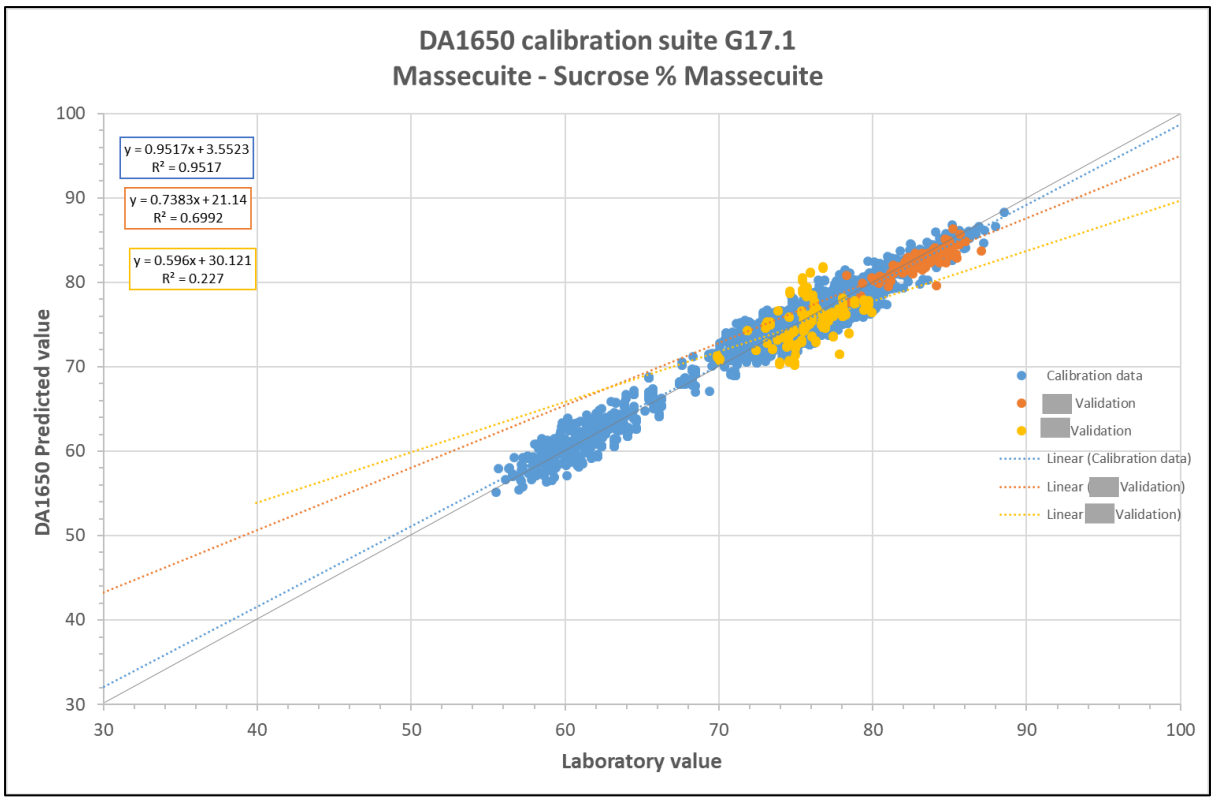

Figure 76: Calibration (blue) and validation of Global 17.1 massecuite sucrose at Mill 2 (orange) and Mill 1 (yellow) in 2017

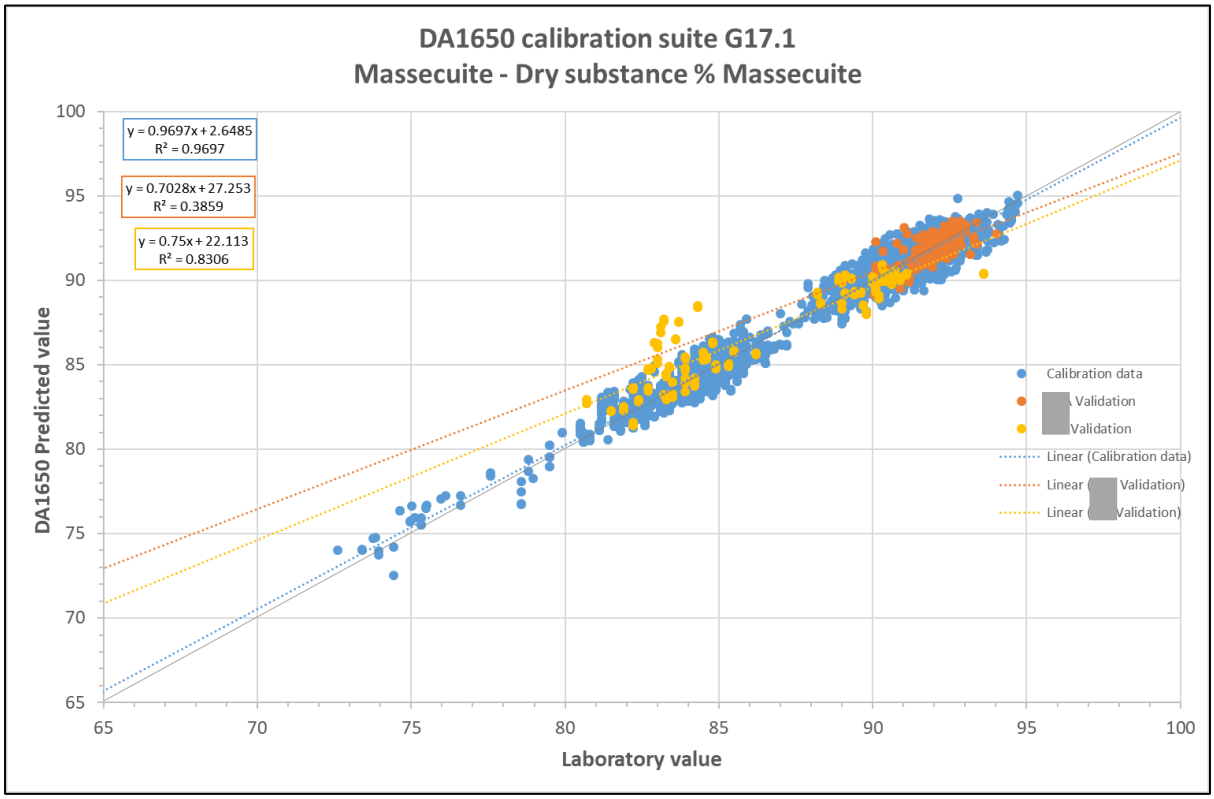

Figure 77: Calibration (blue) and validation of Global 17.1 massecuite dry substance at Mill 2 (orange) and Mill 1 (yellow) in 2017

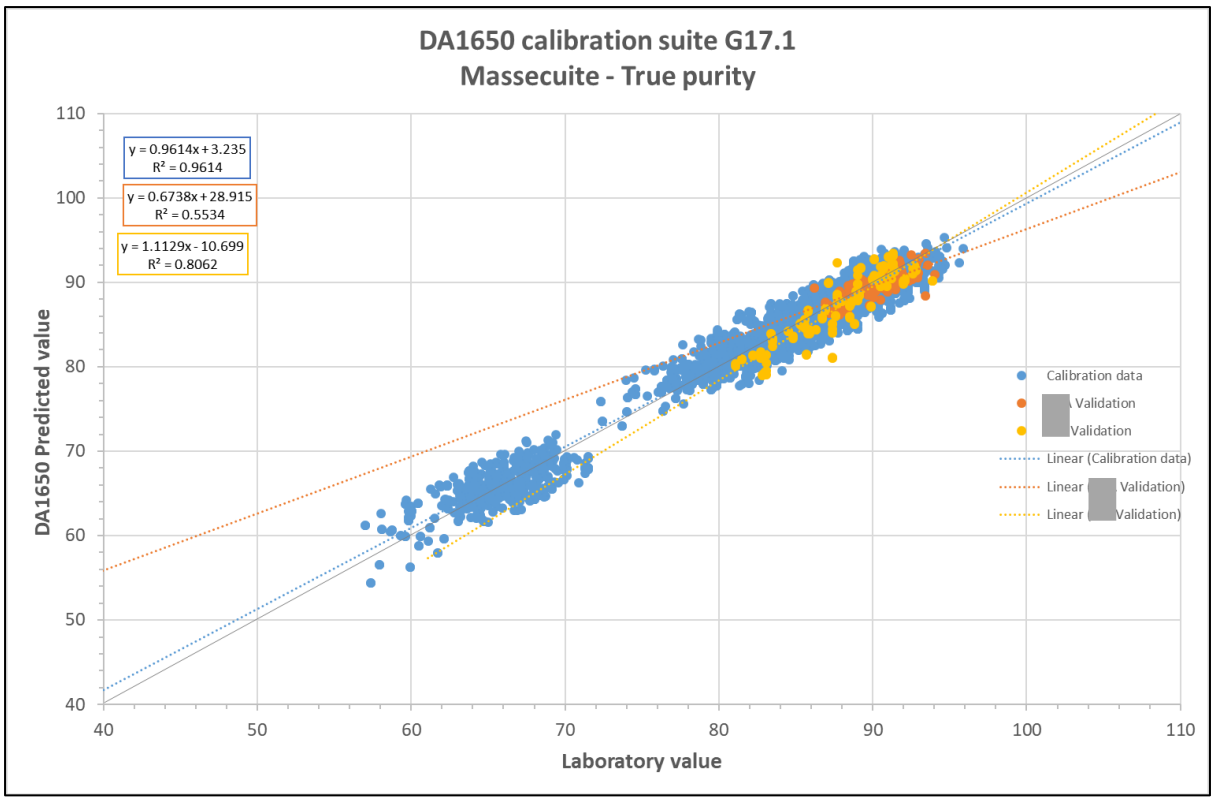

Figure 78: Calibration (blue) and validation of Global 17.1 massecuite true purity at Mill 2 (orange) and Mill 1 (yellow) in 2017

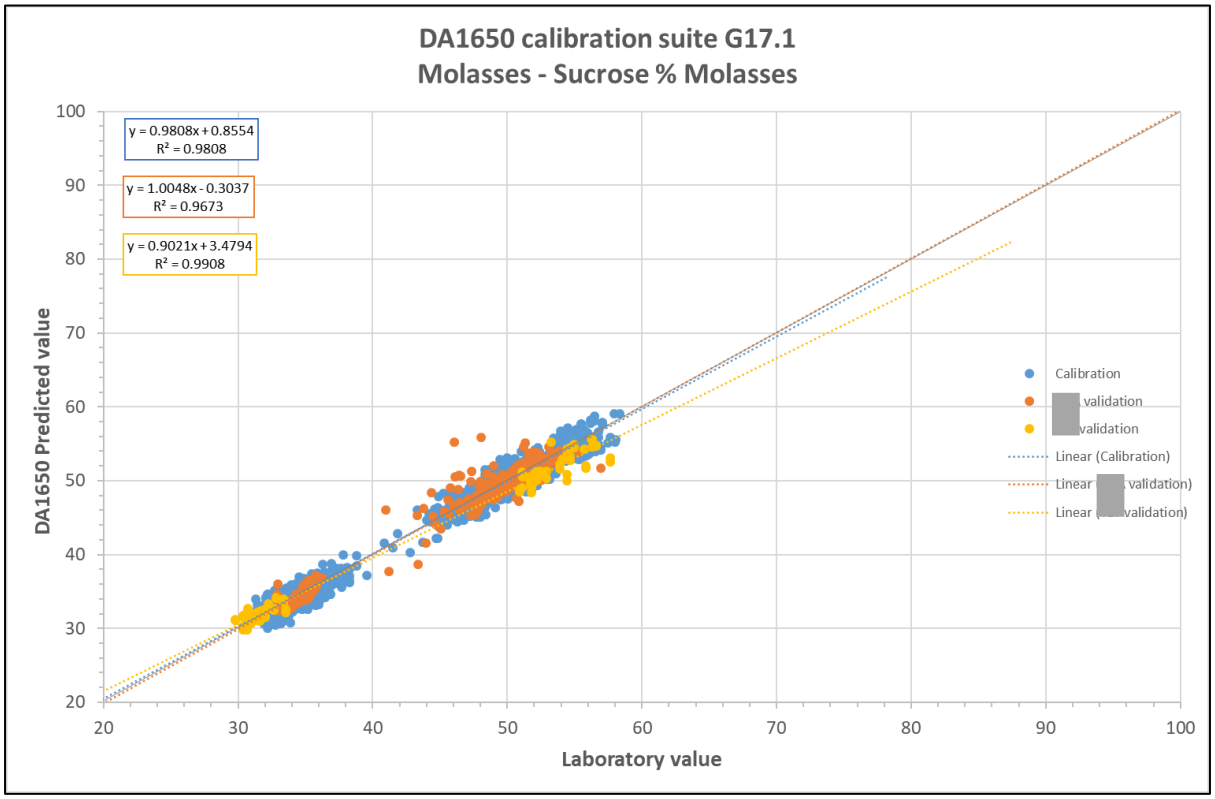

Figure 79: Calibration (blue) and validation of Global 17.1 molasses sucrose at Mill 2 (orange) and Mill 1 (yellow) in 2017

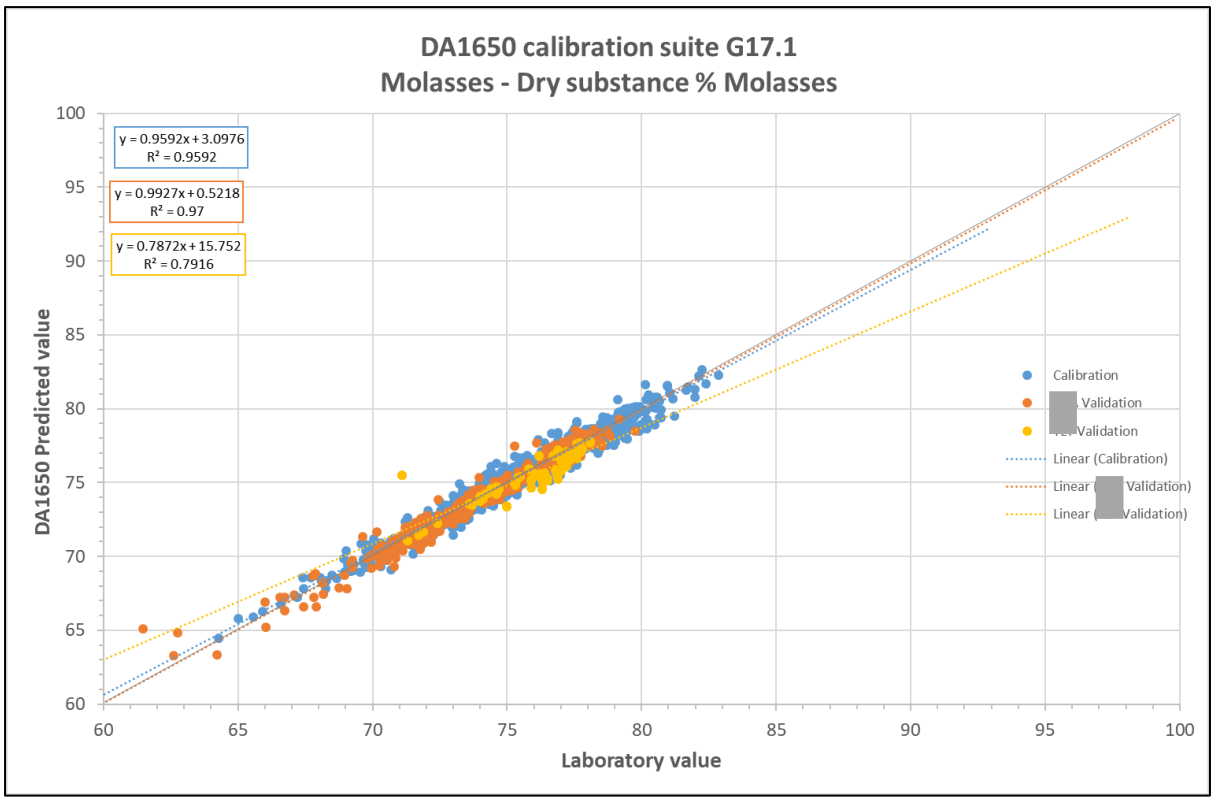

Figure 80: Calibration (blue) and validation of Global 17.1 molasses dry substance at Mill 2 (orange) and Mill 1 (yellow) in 2017

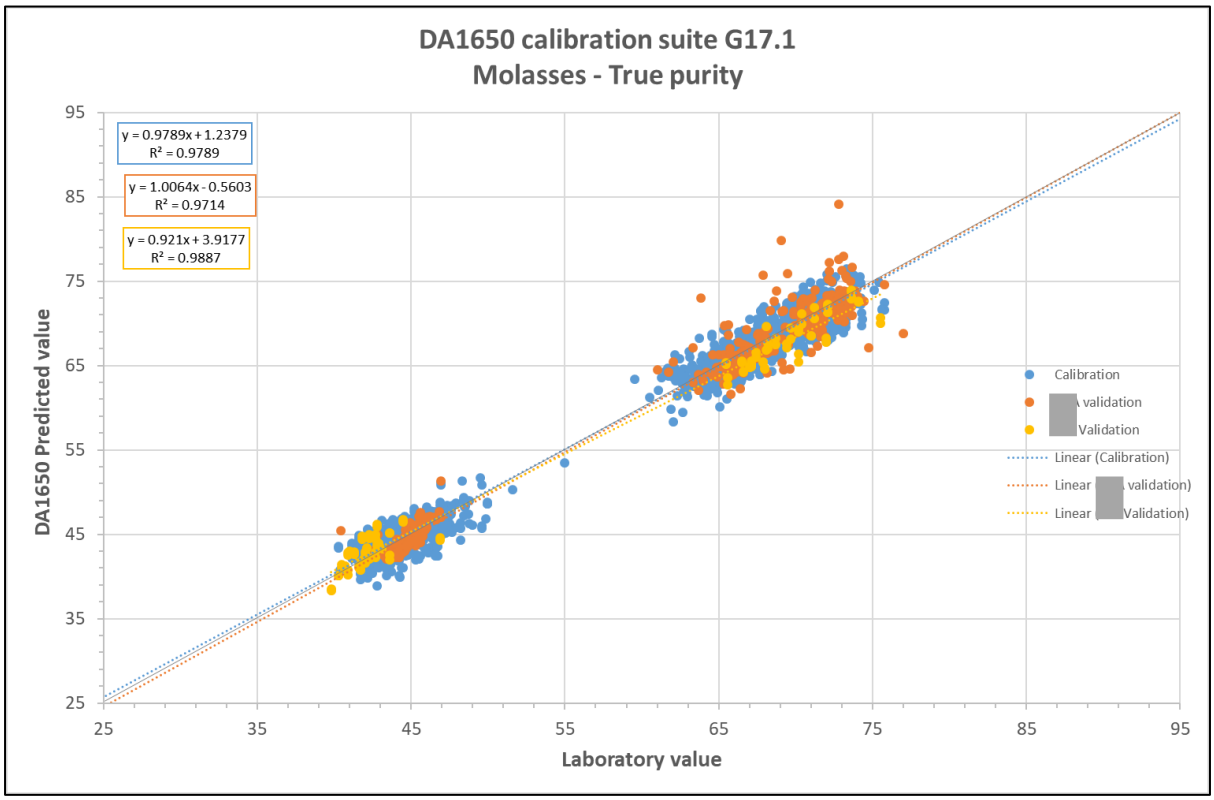

Figure 81: Calibration (blue) and validation of Global 17.1 molasses true purity at Mill 2 (orange) and Mill 1 (yellow) in 2017

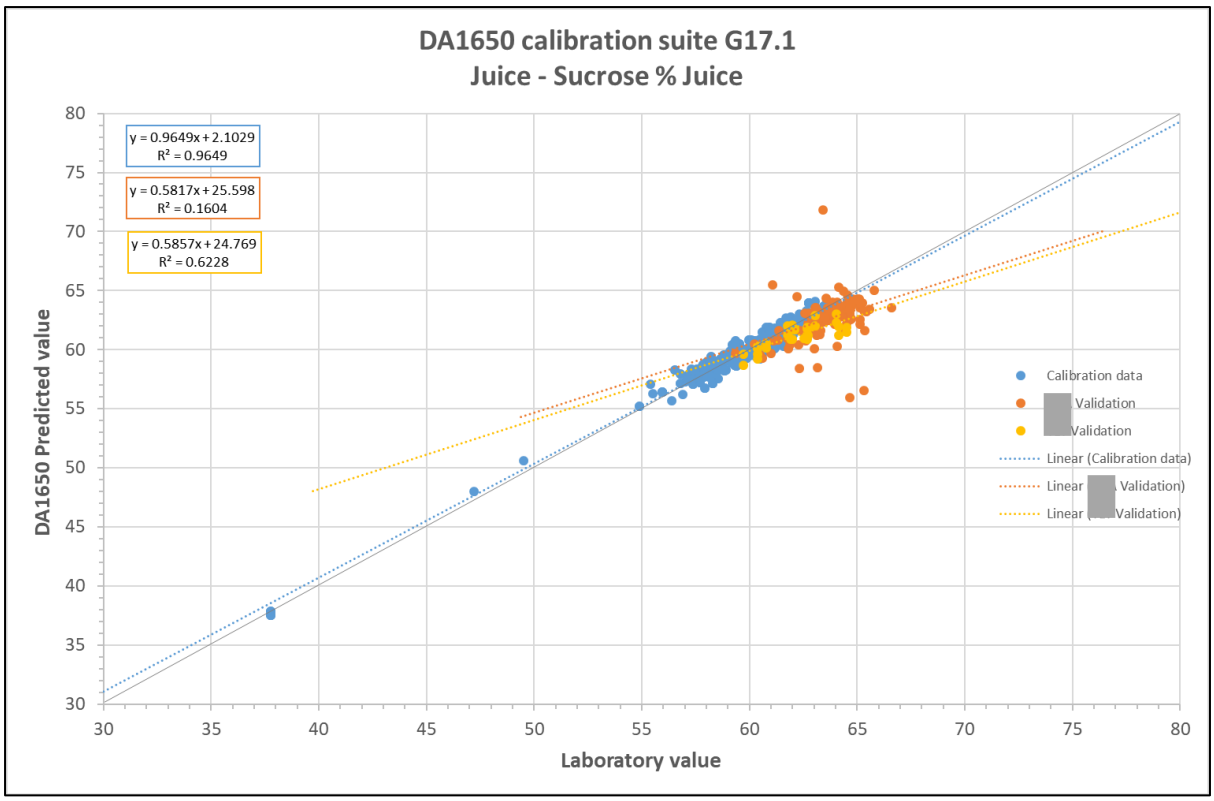

Figure 82: Calibration (blue) and validation of Global 17.1 juice sucrose at Mill 2 (orange) and Mill 1 (yellow) in 2017

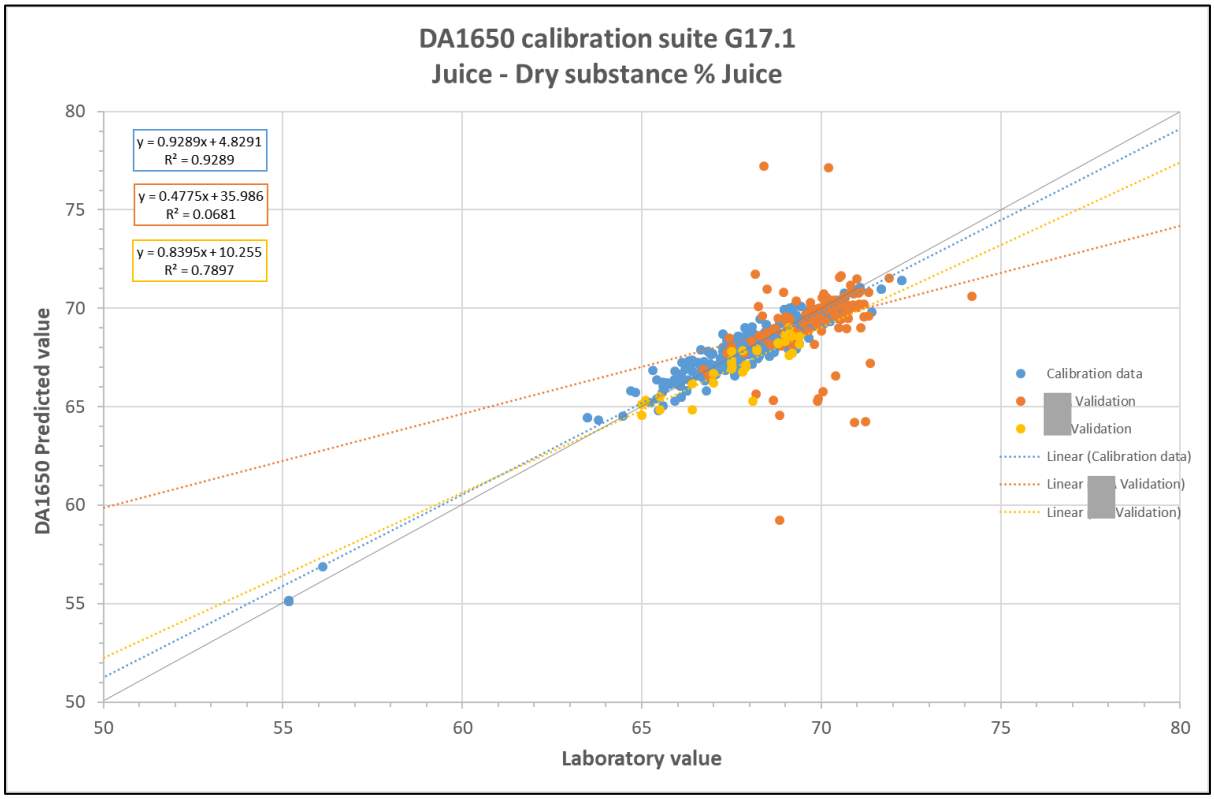

Figure 83: Calibration (blue) and validation of Global 17.1 juice dry substance at Mill 2 (orange) and Mill 1 (yellow) in 2017

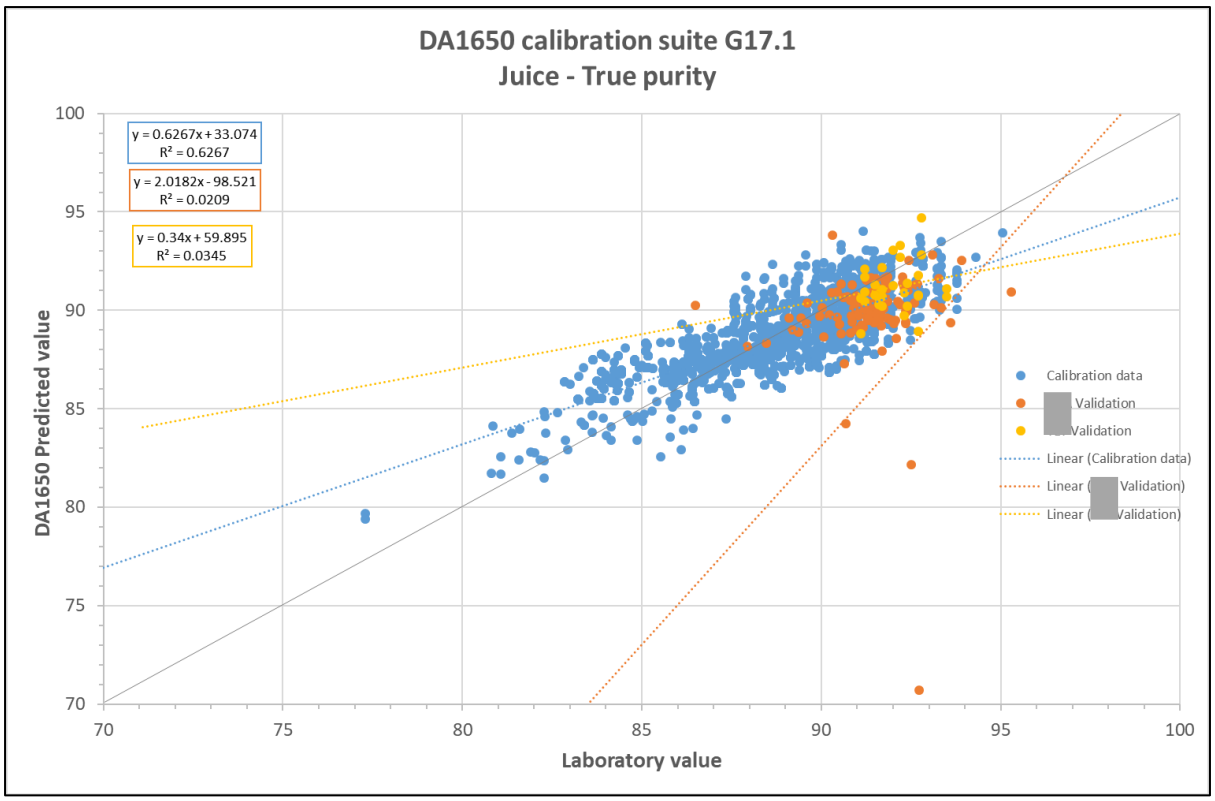

Figure 84: Calibration (blue) and validation of Global 17.1 juice true purity at Mill 2 (orange) and Mill 1 (yellow) in 2017

6.1.4. Method validation

Method validation is the process by which an analytical method is determined to be appropriate for use (Harris, 2007). NIR spectroscopic techniques are not typically evaluated in the same way as the bulk analysis techniques renders many of the analytical assessments inappropriate. Instead, the metrics described in Section 4 of the affiliated industry report *Overview of benchtop near infrared (NIR) spectroscopic applications in the sugarcane mill environment* (Keeffe and Staunton, 2017) are used to evaluate the ongoing performance of the spectral technique. These methods are based on those outlined in ASTM standards D6122-13, E1655, E1866, D6708 and OIML R 135.

This section describes the evaluation of the NIR spectroscopic technique for accuracy, precision, specificity, sensitivity, linearity and range. Where possible, the techniques used for analytical methods were used or tested. If not suitable, alternative methods for evaluating method performance is described.

6.1.4.1. Accuracy

Accuracy is the closeness of agreement between a test result (measurement result) and the accepted reference value (true value). In agriculture, there is no such thing as a duplicate sample and therefore, the true value can never be determined. As an alternative, the true value is typically assumed equivalent to the average value obtained from a large series of tests on a single sample conducted by several certified methods. Often, when evaluating NIR spectroscopic techniques, the reference method is assumed to define the true value. As a single analysis, this is an inappropriate assignment.

If the reference method is to be used to generate a true value, the sample should be analysed in triplicate from the sampling step and the values averaged to define the true value with an associated error margin based on the standard deviation of the group of measurements.

Techniques for evaluating the accuracy of a method include: analysis of a certified reference material, comparing results from two or more different analytical methods, analysing a blank sample spiked with a known amount of analyte, or if a blank sample is not available, analysing the sample by standard addition (Harris, 2007).

As sugarcane factory products readily change with time due to their high moisture and/or sugar concentration, standard reference materials in a similar format to the products analysed in the factory are not available. Similarly, blank samples (e.g. molasses without sucrose) do not exist.

In theory, standard addition would be a suitable technique for evaluating the accuracy of the NIR spectroscopic technique. This process involves adding increasing, known quantities of an analyte to the unknown sample. A linear calibration curve can then be obtained and the unknown concentration calculated. To calculate the concentration of analyte in the original material, the instrument signal from the original and amended products are required, according to the following formula:

$$\frac{[X]_i}{[S]_f + [X]_f} = \frac{I_X}{I_{S+X}}$$

Where: *$[X]_I$ is the initial concentration of the analyte*
$[X]_f$ is the final concentration of the analyte
$[S]_f$ is the concentration of the standard in the final solution
I_X is signal intensity of the initial analyte
I_{S+X} is the signal intensity of the final analyte

This type of analysis is typically used for chromatographic techniques, which produce a single instrument response per analyte, such as peak area or peak height. Due to the high degree of co-variance for each NIR wavelength, change to a single analyte will affect the whole NIR spectrum, rather than a single wavelength. Consequently, a single 'intensity' from the NIR spectrum cannot be used in this formula. In a similar vein to this, samples could be spiked with known concentrations of analyte and the NIR predicted value could be used as the response.

This process was conducted for a selection of analytes in most of the sugar factory products, as described in Table 4. Ideally, the NIR spectroscopic predicted values would reflect the change in concentration of the spiked analyte in proportion to the actual change in concentration. Unfortunately, the data consistently showed that this was not the case. The data were processed in several ways. First, the percentage change of the physical spiking to the sample was calculated based on sample mass, mass of the spiking media and the initial concentration of the spiked analyte (predicted by NIR spectroscopy). This was compared to the percentage change in the NIR predicted values collected for each spike stage. Subsequently, PCA of the predicted wet chemistry values was conducted and evaluated based on the trends associated with the applied spike treatment.

Occasionally, the data showed a linear response, such as that observed for the addition of sucrose to Molasses (Table 16, Figure 85). However, the data typically showed non-linear and variable responses, such as that for the addition of reducing sugars to molasses (Table 17, Figure 86).

Additionally, the NIR predicted values for the other analytes in the sample (the non-spiked sample) tended to behave erratically in the spiked samples (Table 16, Table 17). The NIR calibration is built using a population of samples that contain a known variation of analytes within the matrix. Spiking the sample in a way that is not “typical” of the normal population captured in the calibration will effectively move the product outside the known population captured by the calibration making it a Y-outlier, i.e. in practice, one analyte does not move independently of all the others.

Table 16: Spiking experiment - Evaluation of sucrose in molasses

Mol. weight (g)	Sucrose added (g)	Target Sucrose conc.	Conc. % change	NIR predicted value (%)								% change NIR predicted value							
				Pol	RS	Brix	DS	Suc.	Ash	Acet. acid	Lactic acid	Pol	RS	Brix	DS	Suc.	Ash	Acetic acid	Lactic acid
	0			32.36	12.40	86.25	76.51	31.62	13.01	13.4	66.0								
	0	31.6%	0.0%	32.56	12.64	86.26	76.42	31.48	12.87	12.5	62.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
99.5862	7.8114			36.23	12.53	87.45	78.13	35.73	12.97	8.4	59.1								
99.5862	7.8114	36.5%	15.7%	36.71	12.86	87.57	78.29	35.62	12.80	7.0	54.0	12.4	1.4	1.5	2.3	13.1	-0.4	-40.5	-12.0
102.1764	20.4209			43.62	13.05	89.79	81.34	42.07	12.85	0.6	43.3								
102.1764	20.4209	47.0%	48.9%	44.38	13.36	89.95	81.65	42.60	12.65	-0.9	37.6	35.6	5.5	4.2	6.6	34.2	-1.5	-101.2	-37.0
112.7856	21.5079			49.12	13.75	91.61	83.69	46.90	12.48	-5.0	27.1								
112.7856	21.5079	55.4%	75.6%	49.12	14.11	91.85	83.96	46.87	12.34	-6.0	22.3	51.3	11.3	6.3	9.6	48.6	-4.1	-142.5	-61.6

Mol.: molasses, conc.: concentration, RS: reducing sugars, DS: dry substance, Acet.: acetic, Suc.: sucrose

Table 17: Spiking experiment - Evaluation of reducing sugars in molasses

Mol. weight (g)	Sucrose added (g)	Target Sucrose conc.	Conc. % change	NIR predicted value (%)								% change NIR predicted value							
				Pol	RS	Brix	DS	Suc.	Ash	Acetic acid	Lactic acid	Pol	RS	Brix	DS	Suc.	Ash	Acetic acid	Lactic acid
70	0			77.93	69.8	54.48	9.65	8.55	50.49	6.3	24.7								
70	0			76.85	69.21	54.76	9.85	7.94	53.86	4.3	19								
70	0	54.1%	0.0%	75.79	68.83	53.02	9.42	5.46	54.43	9.9	23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
70.5	0.5			75.61	69.67	50.35	9.23	5.68	52.33	15	22.6								
70.5	0.5			75.25	69.46	49.65	9.11	4.76	52.7	17.4	24.2								
70.5	0.5	54.8%	1.3%	75.64	69.66	49.95	9.21	5.9	52.29	14.9	19.4	-1.8	0.5	-7.6	-4.7	-25.6	-0.9	130.7	-0.7
71	0.5			78.99	71.51	54.08	8.93	7.39	50.83	9.4	7.3								
71	0.5			78.8	71.58	54.87	8.87	7.65	50.72	10	5.3								
71	0.5	55.5%	2.6%	78.22	71.18	52.67	9.24	7.7	51.2	9.4	9.6	2.4	3.1	-0.4	-6.5	3.6	-3.8	40.5	-66.7
72	1			79.58	72.26	55.51	8.69	8.47	51.46	8.9	-6								
72	1			79.9	72.35	55.52	8.51	8.48	50.77	9.2	-3.9								
72	1	56.9%	5.2%	79.59	72.06	54.47	8.52	8.27	50.88	9	0.4	3.7	4.2	2.0	-11.1	14.9	-3.6	32.2	-114.2
74	2			81.2	73.15	55.75	7.62	8.41	51.58	7.8	8.2								
74	2			81.79	73.61	53.05	8.51	9.13	51.16	7.2	18.5								
74	2	59.6%	10.2%	81.29	73.2	54.72	7.85	8.87	51.12	7.5	6.8	5.9	5.8	0.8	-17.1	20.3	-3.1	9.8	-49.8

Mol.: molasses, conc.: concentration, RS: reducing sugars, DS: dry substance, Acet.: acetic, Suc.: sucrose

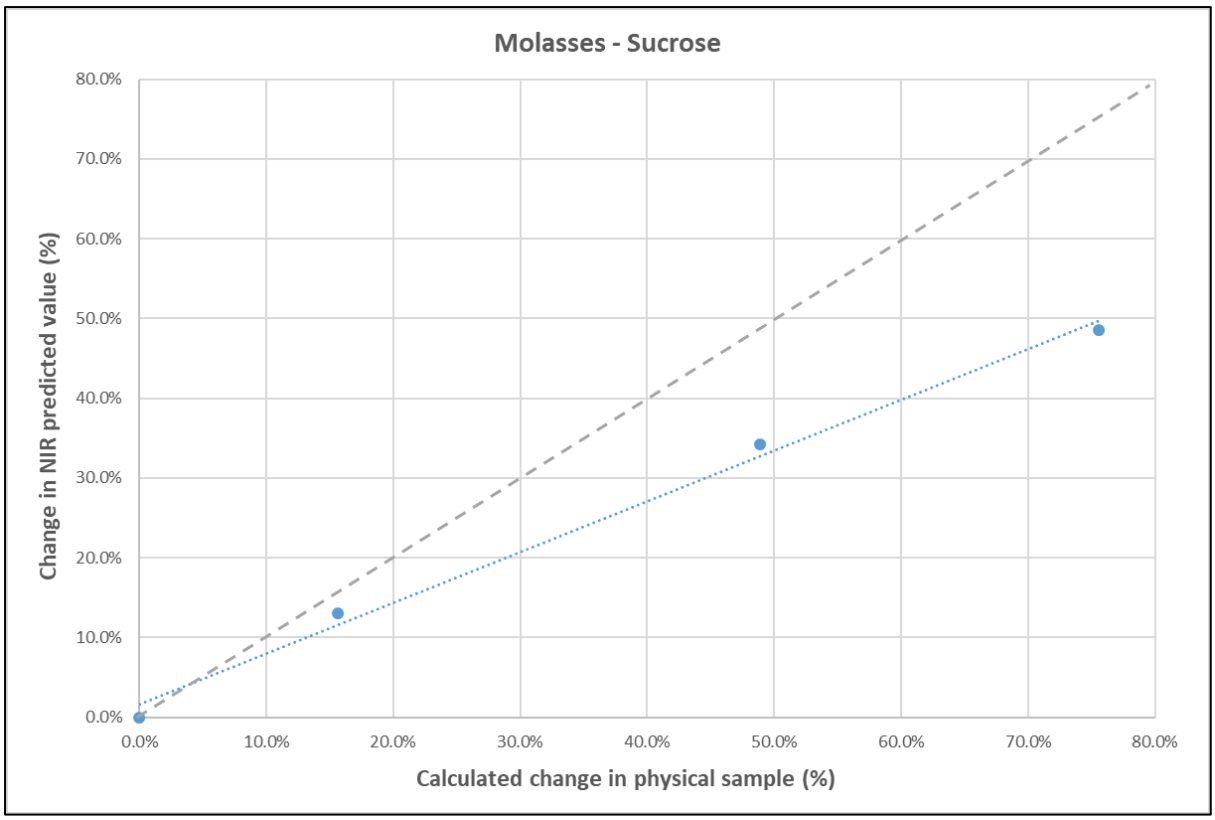

Figure 85: Spiking experiment - Evaluation of sucrose in molasses

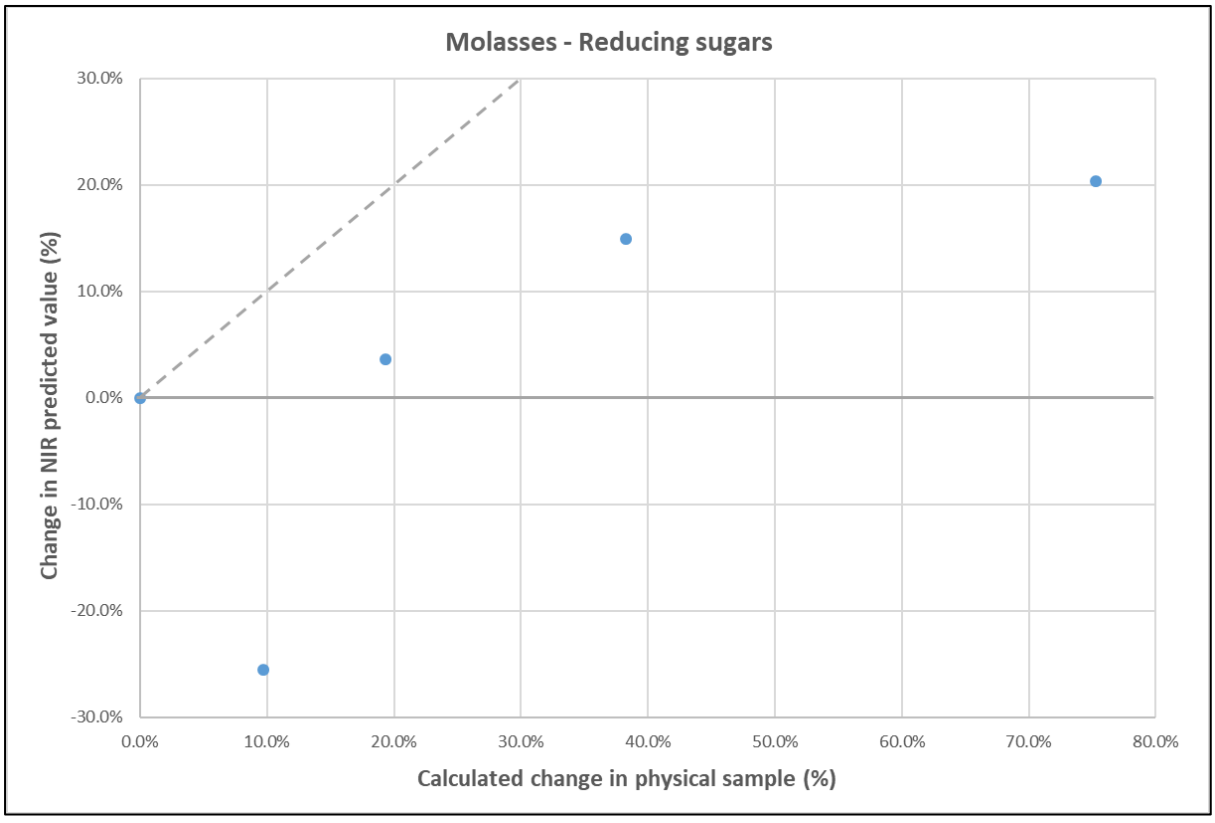

Figure 86: Spiking experiment - Evaluation of reducing sugars in molasses

The whole-matrix effect of spiking samples is best observed with PCA of the NIR predicted values from the different treatments. Typically, the change in the spiked sample was observed in the scores plot as a consistent shift on PC1 or PC2 and the increasing concentration of the analyte correlated with the loading of the target analyte. An example of this is provided in Figure 87 and Figure 88. The scores plot shows the trend from left to right of molasses, with increasing amounts of added $KCl/CaCl_2$ mixture (0.1 g, 0.3 g and 1.3 g). The loadings plot indicates that as the concentration of ash increases with spiking, the predicted values for ash, reducing sugars, dry substance, brix and lactic acid also increase, whereas the sucrose, pol and acetic acid predicted values decrease. This is relatively similar to what is seen for a PCA analysis of different natural molasses samples (Figure 89), except the reducing sugar results are inverted. Figure 90 shows that the loading for reducing sugars is correlated with pol and sucrose for normal samples. This indicated that spiking the sample is affecting the matrix to an extent that the prediction models break down.

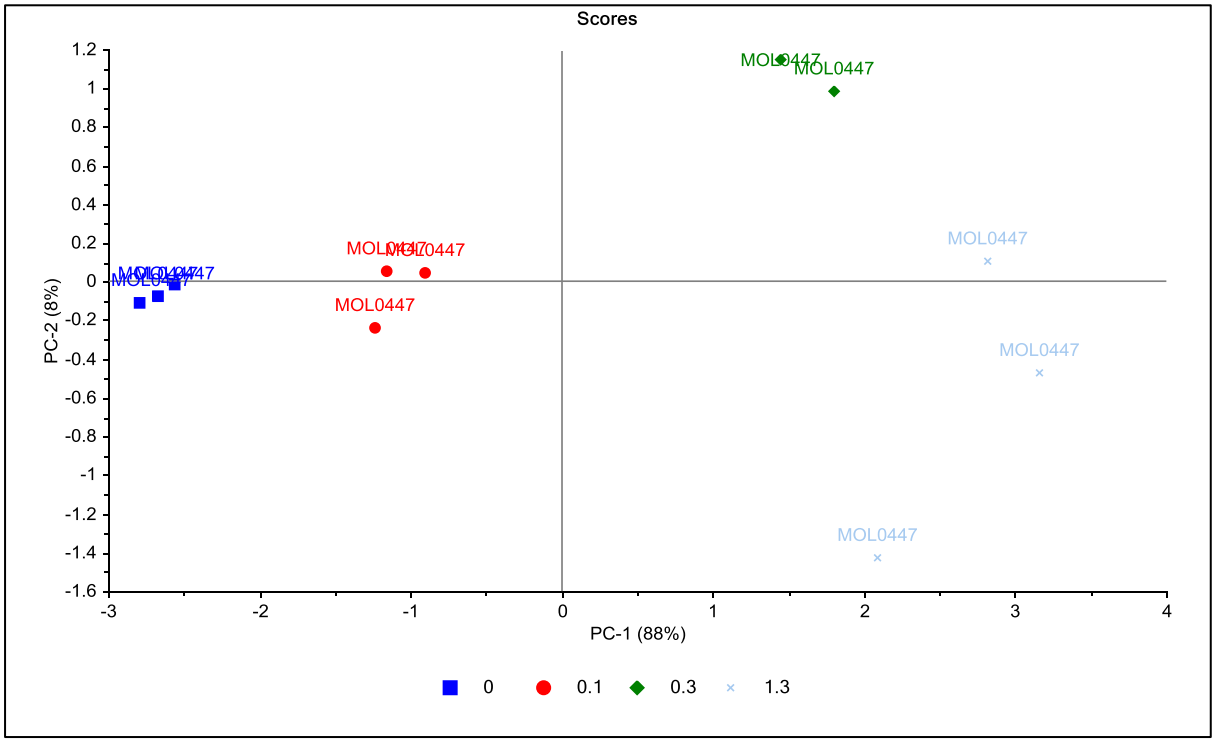

Figure 87: PCA scores plot of spiking experiment – ash in molasses

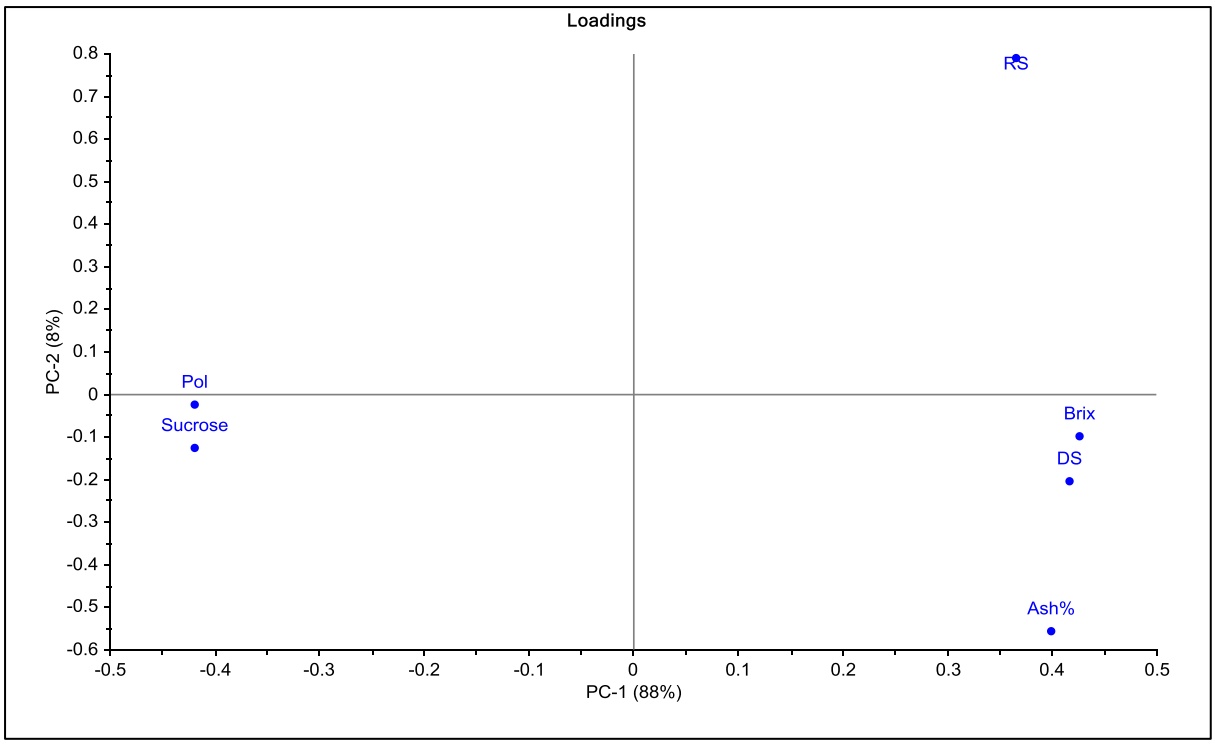

Figure 88: PCA loadings plot of spiking experiment – ash in molasses

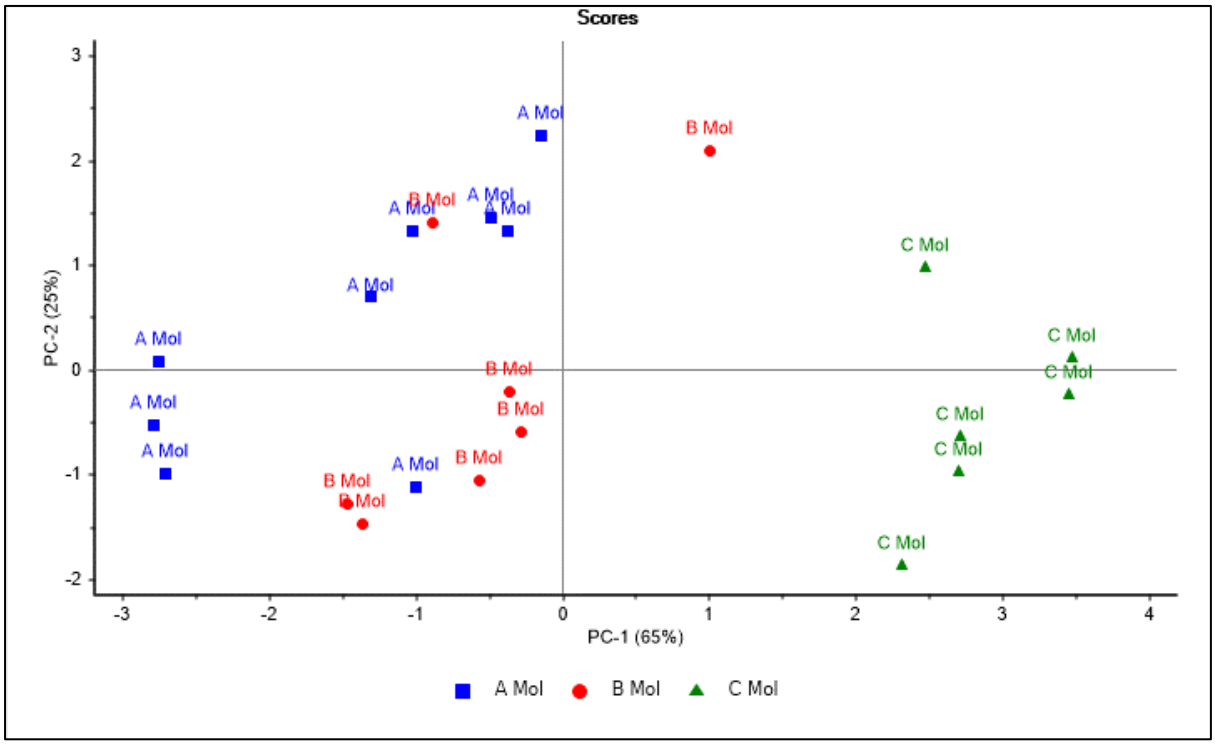

Figure 89: PCA scores plot of NIR predicted values for molasses samples

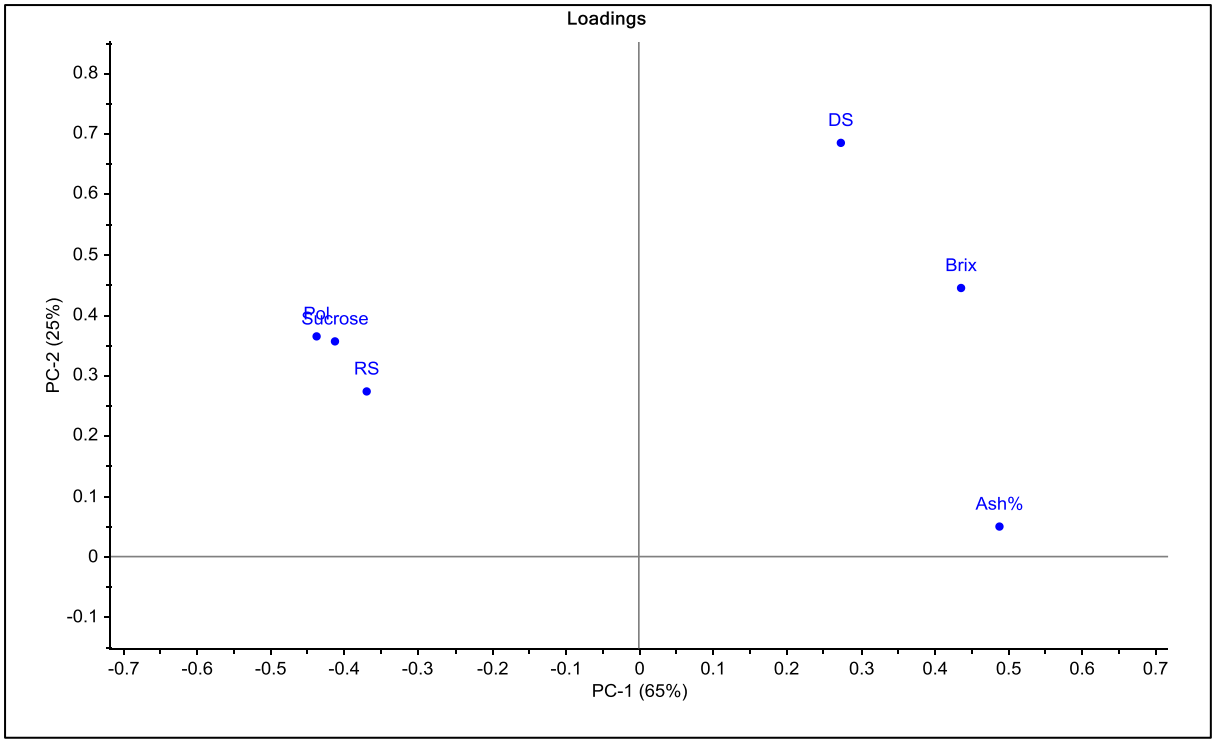

Figure 90: PCA loadings plot of NIR predicted values for molasses samples

The reason that the NIR calibrations do not respond like a standard analytical method to sample spiking is due to its' bulk analysis properties. When the NIR instrument 'sees' an analyte, it does so relative to its surrounding matrix; the hydrogen-bonding between the analyte and its surrounds plays a particularly significant role. When a spike/adulterant analyte is added to a product, it rarely interacts with the sample in the same way as the native analyte, integrated into the sample through a factory process. For example, adding a $KCl/CaCl_2$ mixture to sugar to mimic increasing ash levels results in a coating of the molasses layer of the crystal with discrete, crystalline particles of KCl and $CaCl_2$. There will be minimal interaction between these particles and the original sample matrix. Alternatively, the native K and Ca impurities in the sugar crystal exist as impurities in the crystal lattice of the sucrose and hydrogen-bonded salts or amorphous crystals in the molasses layer. These two presentations of the constituents are chemically different and have different NIR spectra.

This effect is well described in the literature around melamine adulteration of milk (Yakes et al., 2017, Scholl et al., 2017). Scholl et al. demonstrated that milk powder spiked with melamine as a wet blend process (during the production) was difficult to observe with NIR, whereas dry blended melamine (post-process mixing) was easily detected in the NIR spectrum. In dry mixing, melamine is present as a crystalline product on the surface of a milk powder particle (Figure 91 and Figure 92), which both concentrates the contaminant in an area that is 'easy' to observe by NIR (the surface), and provides sharp NIR spectroscopic peaks that are readily interpreted by the human eye and chemometric techniques. Alternatively, wet blending integrates the melamine into the matrix through the formation of lactose/ melamine complexes (Figure 93). The dilution effect, as well as the changes to the hydrogen bonding and covalent bonding of the melamine, rendered the melamine harder to detect by NIR spectroscopy.

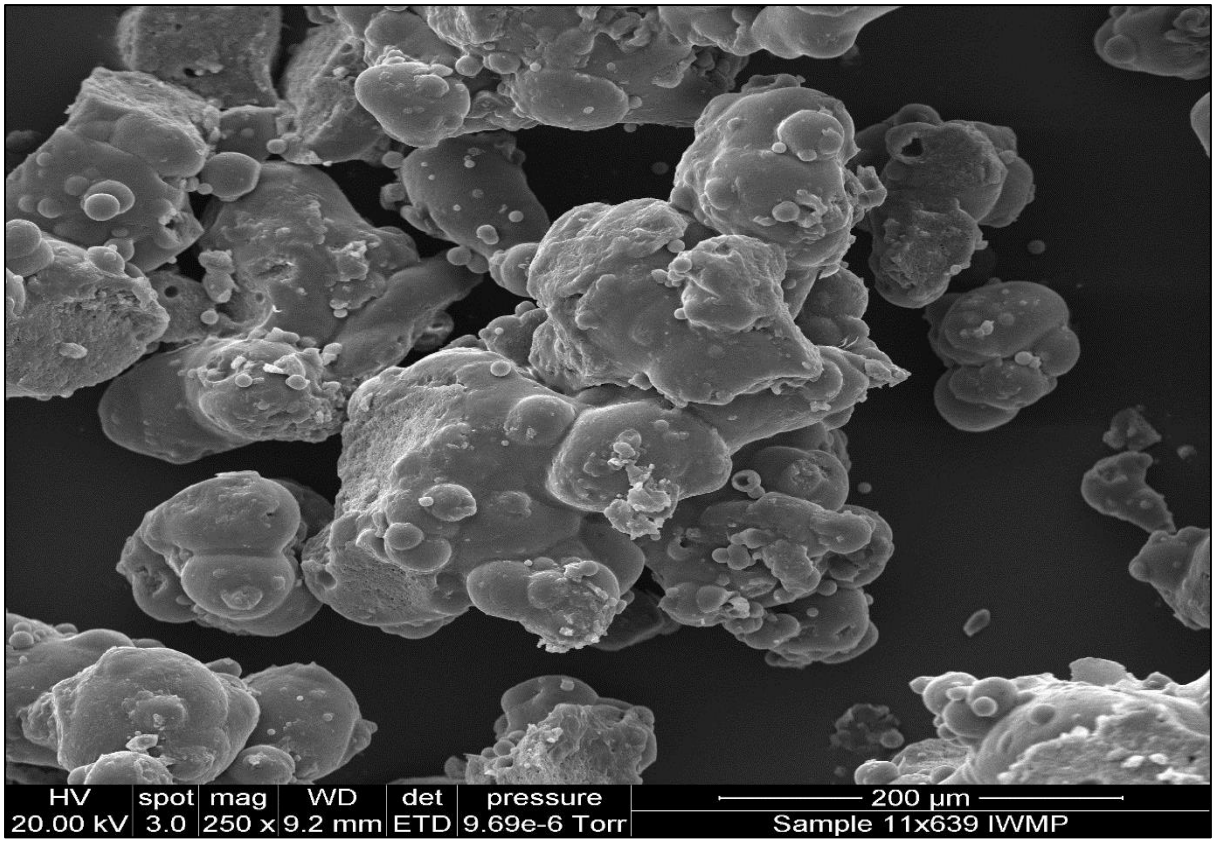

Figure 91: Scanning electron microscopy image of whole milk powder (used with permission from S Holroyd, Fonterra)

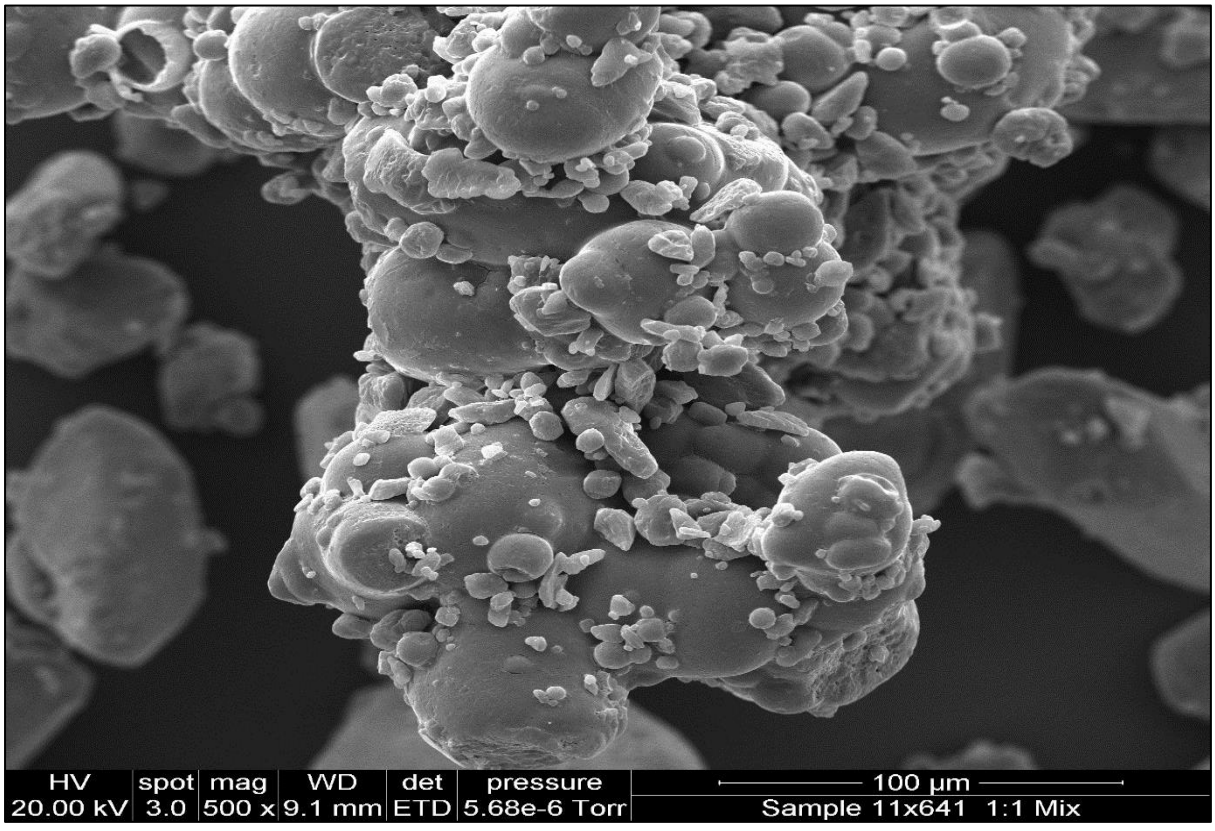

Figure 92: Scanning electron microscopy image of whole milk powder dry-blended with 1 % melamine (used with permission from S Holroyd, Fonterra)

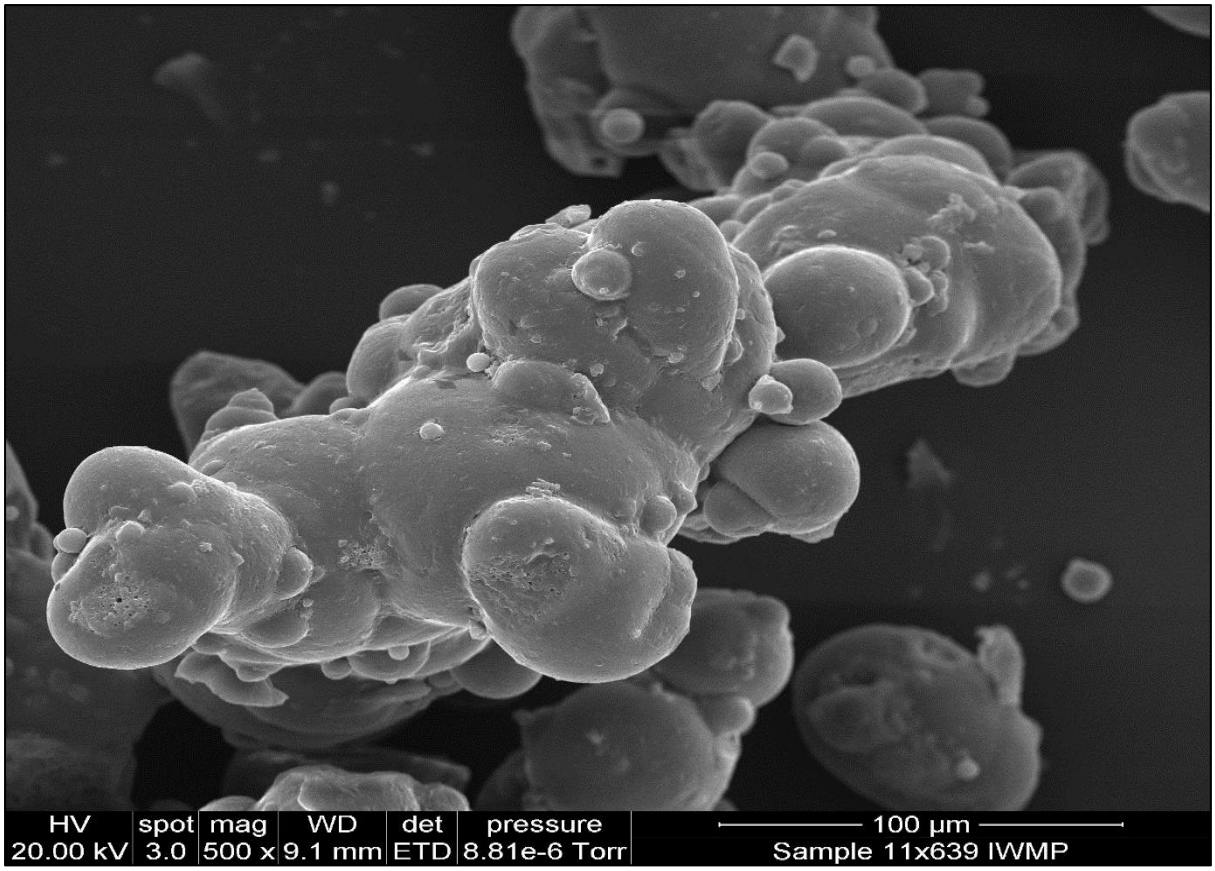

Figure 93: Scanning electron microscopy image of whole milk powder wet-blended with 1 % melamine (used with permission from S Holroyd, Fonterra)

The spectral changes observed due to the interaction of the melamine with the sample matrix affected how the NIR spectrometer 'read' the analyte in the sample. Neither was incorrect, but this process demonstrated the importance of calibrating the system against real formulations, or the formulation in which the technique will be used, rather than manufactured 'standards'. For our factory products, the variance in the spectra of manufactured (spiked) samples and factory-produced (normal) samples will prevent the appropriate prediction from the calibration model due to subtle spectral differences, in the same way.

The final technique suggested by Harris to evaluate accuracy is comparison analysis results by two methods (Harris, 2007). This is a standard procedure used in NIR spectroscopy for evaluating method performance. The NIR predicted values for a number of samples are regressed against the laboratory-derived values and the relationship compared. For agricultural products, a coefficient of determination greater than 0.85 is considered strong, but higher is often achieved. This process can be conducted for the calibration set and an independent validation set to give confidence in the model's ability to predict new samples.

6.1.4.2. Precision

Precision is the closeness of agreement between independent tests results obtained under stipulated conditions.

Repeatability is the estimate of precision under repeatability conditions, where independent test results are obtained with the same method on identical samples in the same laboratory by the same operator using the same equipment within short intervals of time.

Reproducibility is the precision under reproducibility conditions, where test results are obtained with the same method on identical test items under different conditions, including different: operators, equipment, laboratories or time. Reproducibility conditions include variations in one or all of these properties.

Two experiments were conducted to evaluate the precision of the NIR spectroscopic technique. The standard single-sample approach was used for all calibration models and provided the precision statistics outlined in Table 18.

This experiment was based around analysis of a single sample for each product by two operators on two different instruments installed in the same laboratory for the duration of the experiment. Installing two instruments in the same laboratory for a period to conduct repeatability studies was difficult, as those that exist in the industry are heavily utilised. For the first experiment, the SRA and QSL instruments were used.

The QSL instrument is not typically used for sugar factory products and therefore, has not been bias adjusted to account for instrument-specific variation. Consequently, a considerable bias existed between these two instruments. To account for this, a bias was estimated based on the average difference between the QSL and SRA instruments and was applied to the QSL-predicted data. The precision statistics were re-calculated based on these results, labelled SRA and QSL B in Table 18. The statistics were also calculated on the SRA data only (between analyst reproducibility). For most parameters, the reproducibility between the SRA only and bias adjusted QSL datasets were similar. This indicates that the bias adjustments did not provide erroneous results, but better reflect a tuned instrument.

For comparison, the published precision data for a selection of comparable methods from the Australian (2001b) and international (Bartens, 2011) sugar laboratory methods books are provided inTable 20. Australian sugar mills do not routinely conduct ring testing or have reproducibility data available for their standard methods and conditions. Most mills operate using methods that have been modified from the ICUMSA or BSES methods, or developed in house. Each variation contributes differently to the error profile of the method. Consequently, a method's published repeatability or reproducibility is not equivalent to a particular laboratory, or group of laboratories' performance, however it is indicative of the expected performance under optimal conditions. Due to the difficulty in undertaking ring tests to obtain precision data for methods, several of the published methods do not have repeatability and/or reproducibility values ascribed.

A comparison of the repeatability and reproducibility data from the NIR spectroscopy trials in Table 18 and those published in comparable wet chemistry methods demonstrates that the performance of the NIR spectroscopic technique meets or exceeds the quality metrics of the wet chemistry methods. Where reproducibility values are available for the wet chemistry, they are larger than the respective value for the NIR spectroscopic technique, where bias between the two instruments has been accounted for.

In addition to the single-sample precision experiments, a population-based experiment for raw sugar was conducted, as this calibration is mature. The precision statistics for this experiment are provided in Table 19. Similarly to the single-sample experiments, the precision values for the NIR spectroscopic method outperform the published values for the official methods.

Table 18: Precision metrics for single sample experiments

			n	Outl.	Average	RPBLTY SD (S_r)	RPDCBLT SD (S_R)	Relative SD(r)	Relative SD(R)	RPBLTY (r)	RPDCBLT (R)	Standard Uncert. (u)	Expanded Uncert. (U)	Bias adjust.
Final mill bagasse	Moisture	SRA and QSL	4	0	43.23	0.577	1.075	1.336	2.488	1.155	2.151	1.075	2.151	
		SRA and QSL B	4	0	43.58	0.577	0.999	1.325	2.293	1.155	1.999	0.999	1.999	0.69
		SRA only	2	0	43.58	0.619	1.173	1.419	2.692	1.237	2.346	1.659	3.318	
	Pol	SRA and QSL	4	0	1.90	0.175	0.308	9.251	16.253	0.351	0.616	0.308	0.616	
		SRA and QSL B	4	0	2.11	0.175	0.177	8.296	8.396	0.351	0.355	0.177	0.355	0.44
		SRA only	2	0	2.11	0.235	0.255	11.100	12.078	0.469	0.511	0.361	0.722	
	Brix	SRA and QSL	4	0	1.09	0.009	0.350	0.798	32.094	0.017	0.701	0.350	0.701	
		SRA and QSL B	4	0	1.39	0.009	0.015	0.624	1.089	0.017	0.030	0.015	0.030	0.61
		SRA only	2	0	1.39	0.007	0.012	0.511	0.828	0.014	0.023	0.016	0.033	
	Fibre	SRA and QSL	4	0	42.52	0.806	14.624	1.897	34.390	1.613	29.247	14.624	29.247	
		SRA and QSL B	4	0	55.15	0.806	1.137	1.462	2.062	1.613	2.275	1.137	2.275	25.25
		SRA only	2	0	55.15	0.965	1.313	1.751	2.380	1.931	2.625	1.856	3.713	
Syrup	Ash	SRA and QSL	4	1	2.17	0.862	0.861	39.660	39.621	1.724	1.723	0.861	1.723	
		SRA and QSL B	4	1	2.04	0.862	0.843	42.225	41.280	1.724	1.686	0.843	1.686	-0.27
		SRA only	2	1	2.05	0.883	0.862	43.145	42.117	1.767	1.724	1.219	2.439	
	Pol	SRA and QSL	4	0	62.10	2.012	2.134	3.241	3.437	4.025	4.268	2.134	4.268	
		SRA and QSL B	4	0	61.53	2.012	2.032	3.270	3.302	4.025	4.064	2.032	4.064	-1.13
		SRA only	2	0	61.53	2.290	2.304	3.722	3.745	4.581	4.609	3.259	6.518	
	Reducing sugars	SRA and QSL	4	0	2.10	0.158	0.161	7.518	7.671	0.315	0.322	0.161	0.322	
		SRA and QSL B	4	0	2.09	0.158	0.160	7.557	7.688	0.315	0.321	0.160	0.321	-0.02
		SRA only	2	0	2.09	0.112	0.127	5.346	6.072	0.223	0.254	0.179	0.359	
	Dry substance	SRA and QSL	4	0	68.27	1.232	1.409	1.804	2.063	2.464	2.817	1.409	2.817	
		SRA and QSL B	4	0	68.49	1.232	1.387	1.799	2.025	2.464	2.773	1.387	2.773	0.43
		SRA only	2	0	68.49	0.509	0.621	0.743	0.906	1.017	1.242	0.878	1.756	
	Sucrose	SRA and QSL	4	0	63.22	1.049	2.543	1.660	4.022	2.099	5.085	2.543	5.085	
		SRA and QSL B	4	0	61.31	1.049	1.248	1.712	2.036	2.099	2.496	1.248	2.496	-3.84
		SRA only	2	0	61.31	0.882	0.977	1.439	1.594	1.765	1.955	1.382	2.765	
	True purity	SRA and QSL	4	1	89.45	2.170	3.368	2.426	3.765	4.340	6.736	3.368	6.736	
		SRA and QSL B	4	1	87.38	2.170	2.437	2.483	2.789	4.340	4.874	2.437	4.874	-3.99
		SRA only	2	1	87.33	0.694	1.080	0.795	1.237	1.388	2.160	1.528	3.055	

Table 18: Precision metrics for single sample experiments cont.

			n	Outl.	Average	RPBLTY SD (S_r)	RPDCBLT SD (S_R)	Relative SD(r)	Relative SD(R)	RPBLTY (r)	RPDCBLT (R)	Standard Uncert. (u)	Expanded Uncert. (U)	Bias adjust.
Syrup	Brix	SRA and QSL	4	0	67.17	1.148	1.442	1.710	2.147	2.297	2.885	1.442	2.885	
		SRA and QSL B	4	0	66.72	1.148	1.345	1.721	2.016	2.297	2.690	1.345	2.690	-0.90
		SRA only	2	0	66.72	1.253	1.491	1.877	2.235	2.505	2.982	2.109	4.218	
A Molasses	Ash	SRA and QSL	4	0	9.66	0.105	0.299	1.086	3.091	0.210	0.597	0.299	0.597	
		SRA and QSL B	4	0	9.41	0.105	0.097	1.114	1.030	0.210	0.194	0.097	0.194	-0.49
		SRA only	2	0	9.41	0.086	0.074	0.909	0.787	0.171	0.148	0.105	0.209	
	Pol	SRA and QSL	4	0	54.74	0.220	1.154	0.403	2.109	0.441	2.309	1.154	2.309	
		SRA and QSL B	4	0	55.72	0.220	0.223	0.396	0.400	0.441	0.446	0.223	0.446	1.96
		SRA only	2	0	55.72	0.186	0.196	0.335	0.352	0.373	0.392	0.277	0.555	
	Reducing sugars	SRA and QSL	4	0	13.79	0.249	0.254	1.809	1.840	0.499	0.507	0.254	0.507	
		SRA and QSL B	4	0	13.74	0.249	0.247	1.815	1.799	0.499	0.495	0.247	0.495	-0.10
		SRA only	2	0	13.74	0.170	0.120	1.239	0.875	0.340	0.241	0.170	0.340	
	Dry substance	SRA and QSL	4	0	75.99	0.073	0.138	0.096	0.182	0.146	0.277	0.138	0.277	
		SRA and QSL B	4	0	76.07	0.073	0.097	0.096	0.128	0.146	0.195	0.097	0.195	0.17
		SRA only	2	0	76.07	0.078	0.061	0.102	0.080	0.156	0.121	0.086	0.171	
	Sucrose	SRA and QSL	4	0	54.30	0.237	1.616	0.436	2.977	0.474	3.233	1.616	3.233	
		SRA and QSL B	4	0	52.91	0.237	0.203	0.448	0.383	0.474	0.406	0.203	0.406	-2.78
		SRA only	2	0	52.91	0.158	0.155	0.298	0.292	0.315	0.309	0.219	0.437	
	True Purity	SRA and QSL	4	0	3.96	0.679	1.567	17.150	39.607	1.357	3.134	1.567	3.134	
		SRA and QSL B	4	0	2.88	0.679	0.951	23.578	33.044	1.357	1.902	0.951	1.902	-2.16
		SRA only	2	0	2.88	0.407	0.499	14.146	17.351	0.814	0.999	0.706	1.413	
	Brix	SRA and QSL	4	0	81.19	0.072	0.066	0.088	0.081	0.144	0.132	0.066	0.132	
		SRA and QSL B	4	0	81.19	0.072	0.066	0.088	0.081	0.144	0.132	0.066	0.132	-0.01
		SRA only	2	0	81.19	0.048	0.039	0.060	0.048	0.097	0.078	0.055	0.110	
	Acetic	SRA and QSL	4	0	3.96	0.679	1.567	17.150	39.607	1.357	3.134	1.567	3.134	
		SRA and QSL B	4	0	2.88	0.679	0.951	23.578	33.044	1.357	1.902	0.951	1.902	-2.16
		SRA only	2	0	2.88	0.407	0.499	14.146	17.351	0.814	0.999	0.706	1.413	
	Lactic	SRA and QSL	4	2	13.26	2.823	3.005	21.284	22.654	5.646	6.010	3.005	6.010	
		SRA and QSL B	4	2	14.69	2.823	2.274	19.219	15.479	5.646	4.547	2.274	4.547	3.09
		SRA only	2	2	14.85	3.291	2.239	22.163	15.080	6.582	4.478	3.167	6.333	

Table 18: Precision metrics for single sample experiments cont.

			n	Outl.	Average	RPBLTY SD (Sr)	RPDCBLT SD (SR)	Relative SD(r)	Relative SD(R)	RPBLTY (r)	RPDCBLT (R)	Standard Uncert. (u)	Expanded Uncert. (U)	Bias adjust.
Mud	Moisture	SRA and QSL	4	0	72.62	0.421	1.789	0.580	2.463	0.843	3.578	1.789	3.578	
		SRA and QSL B	4	0	73.88	0.421	1.043	0.570	1.412	0.843	2.086	1.043	2.086	2.52
		SRA only	2	0	73.88	0.447	1.043	0.605	1.412	0.895	2.087	1.475	2.951	
	Pol	SRA and QSL	4	0	0.48	0.171	0.266	35.806	55.880	0.341	0.533	0.266	0.533	
		SRA and QSL B	4	0	0.55	0.171	0.254	31.250	46.506	0.341	0.508	0.254	0.508	0.14
		SRA only	2	0	0.55	0.234	0.402	42.867	73.560	0.468	0.804	0.568	1.137	
	Fibre	SRA and QSL	4	0	4.96	0.141	3.363	2.853	67.837	0.283	6.725	3.363	6.725	
		SRA and QSL B	4	0	7.86	0.141	0.277	1.800	3.523	0.283	0.554	0.277	0.554	5.80
		SRA only	2	0	7.86	0.195	0.459	2.486	5.840	0.391	0.918	0.649	1.298	
	Mud Solids	SRA and QSL	4	0	3.46	0.109	2.299	3.143	66.464	0.217	4.597	2.299	4.597	
		SRA and QSL B	4	0	1.48	0.109	0.280	7.331	18.871	0.217	0.560	0.280	0.560	-3.95
		SRA only	2	0	1.48	0.064	0.100	4.303	6.716	0.128	0.199	0.141	0.282	
	Total Insolubles	SRA and QSL	4	0	16.68	0.149	10.341	0.892	62.004	0.297	20.681	10.341	20.681	
		SRA and QSL B	4	0	25.63	0.149	0.357	0.580	1.392	0.297	0.714	0.357	0.714	17.90
		SRA only	2	0	25.63	0.159	0.306	0.620	1.192	0.318	0.611	0.432	0.864	
	Fibre: Mud Solids	SRA and QSL	4	0	0.97	0.046	0.597	4.796	61.656	0.093	1.194	0.597	1.194	
		SRA and QSL B	4	0	1.48	0.046	0.064	3.133	4.326	0.093	0.128	0.064	0.128	1.03
		SRA only	2	0	1.48	0.064	0.100	4.303	6.716	0.128	0.199	0.141	0.282	
Prepared cane	Ash	SRA and QSL	4	0	9.66	0.105	0.299	1.086	3.091	0.210	0.597	0.299	0.597	
		SRA and QSL B	4	0	9.41	0.105	0.097	1.114	1.030	0.210	0.194	0.097	0.194	-0.49
		SRA only	2	0	9.41	0.086	0.074	0.909	0.787	0.171	0.148	0.105	0.209	
	Moisture	SRA and QSL	4	0	54.74	0.220	1.154	0.403	2.109	0.441	2.309	1.154	2.309	
		SRA and QSL B	4	0	55.72	0.220	0.223	0.396	0.400	0.441	0.446	0.223	0.446	1.96
		SRA only	2	0	55.72	0.186	0.196	0.335	0.352	0.373	0.392	0.277	0.555	
	Pol	SRA and QSL	4	0	13.79	0.249	0.254	1.809	1.840	0.499	0.507	0.254	0.507	
		SRA and QSL B	4	0	13.74	0.249	0.247	1.815	1.799	0.499	0.495	0.247	0.495	-0.10
		SRA only	2	0	13.74	0.170	0.120	1.239	0.875	0.340	0.241	0.170	0.340	
	Brix	SRA and QSL	4	0	75.99	0.073	0.138	0.096	0.182	0.146	0.277	0.138	0.277	
		SRA and QSL B	4	0	76.07	0.073	0.097	0.096	0.128	0.146	0.195	0.097	0.195	0.17
		SRA only	2	0	76.07	0.078	0.061	0.102	0.080	0.156	0.121	0.086	0.171	

Table 18: Precision metrics for single sample experiments cont.

			n	Outl.	Average	RPBLTY SD (Sr)	RPDCBLT SD (SR)	Relative SD(r)	Relative SD(R)	RPBLTY (r)	RPDCBLT (R)	Standard Uncert. (u)	Expanded Uncert. (U)	Bias adjust.
Prepared cane	Fibre	SRA and QSL	4	0	54.30	0.237	1.616	0.436	2.977	0.474	3.233	1.616	3.233	
		SRA and QSL B	4	0	52.91	0.237	0.203	0.448	0.383	0.474	0.406	0.203	0.406	-2.78
		SRA only	2	0	52.91	0.158	0.155	0.298	0.292	0.315	0.309	0.219	0.437	
	Brix in cane	SRA and QSL	4	0	3.96	0.679	1.567	17.150	39.607	1.357	3.134	1.567	3.134	
		SRA and QSL B	4	0	2.88	0.679	0.951	23.578	33.044	1.357	1.902	0.951	1.902	-2.16
		SRA only	2	0	2.88	0.407	0.499	14.146	17.351	0.814	0.999	0.706	1.413	
	Pol in cane	SRA and QSL	4	0	81.19	0.072	0.066	0.088	0.081	0.144	0.132	0.066	0.132	
		SRA and QSL B	4	0	81.19	0.072	0.066	0.088	0.081	0.144	0.132	0.066	0.132	-0.01
		SRA only	2	0	81.19	0.048	0.039	0.060	0.048	0.097	0.078	0.055	0.110	
	CCS	SRA and QSL	4	1	3.80	0.436	1.204	11.462	31.685	0.871	2.408	1.204	2.408	
		SRA and QSL B	4	1	3.04	0.436	0.612	14.315	20.109	0.871	1.224	0.612	1.224	-1.57
		SRA only	2	1	2.88	0.407	0.499	14.146	17.351	0.814	0.999	0.706	1.413	
	Dry Matter	SRA and QSL	4	3	12.99	2.565	2.659	19.755	20.472	5.131	5.317	2.659	5.317	
		SRA and QSL B	4	3	14.24	2.565	2.028	18.012	14.236	5.131	4.055	2.028	4.055	2.62
		SRA only	2	3	14.43	2.837	1.739	19.656	12.050	5.675	3.479	2.460	4.920	
Raw sugar	Ash	SRA and QSL	4	0	0.17	0.014	0.023	8.070	13.433	0.028	0.047	0.023	0.047	
		SRA and QSL B	4	0	0.19	0.014	0.019	7.550	10.121	0.028	0.038	0.019	0.038	0.02
		SRA only	2	0	0.19	0.012	0.026	6.622	13.935	0.025	0.052	0.037	0.073	
	Colour	SRA and QSL	4	0	1302	90.64	248.49	6.96	19.08	181.28	496.99	248.49	496.99	
		SRA and QSL B	4	0	1502	90.64	92.37	6.03	6.15	181.28	184.75	92.37	184.75	399.56
		SRA only	2	0	1502	57.06	53.53	3.80	3.56	114.12	107.06	75.71	151.41	
	Dextran	SRA and QSL	4	0	63.41	5.600	12.564	8.831	19.813	11.199	25.127	12.564	25.127	
		SRA and QSL B	4	0	72.12	5.600	7.531	7.764	10.443	11.199	15.062	7.531	15.062	17.42
		SRA only	2	0	72.12	2.796	6.911	3.876	9.582	5.591	13.822	9.773	19.547	
	Filtrability	SRA and QSL	4	0	73.17	1.584	10.010	2.165	13.679	3.169	20.019	10.010	20.019	
		SRA and QSL B	4	0	64.67	1.584	1.924	2.450	2.975	3.169	3.848	1.924	3.848	-17.01
		SRA only	2	0	64.67	1.781	2.149	2.755	3.323	3.563	4.297	3.039	6.077	
	Moisture	SRA and QSL	4	0	0.20	0.014	0.017	7.261	8.830	0.029	0.035	0.017	0.035	
		SRA and QSL B	4	0	0.20	0.014	0.017	7.160	8.557	0.029	0.034	0.017	0.034	0.01
		SRA only	2	0	0.20	0.014	0.023	6.879	11.487	0.028	0.046	0.033	0.065	

Table 18: Precision metrics for single sample experiments cont.

			n	Outl.	Average	RPBLTY SD (Sr)	RPDCBLT SD (SR)	Relative SD(r)	Relative SD(R)	RPBLTY (r)	RPDCBLT (R)	Standard Uncert. (u)	Expanded Uncert. (U)	Bias adjust.
Raw sugar	Pol	SRA and QSL	4	0	99.23	0.079	0.139	0.080	0.140	0.158	0.279	0.139	0.279	
		SRA and QSL B	4	0	99.33	0.079	0.076	0.080	0.077	0.158	0.152	0.076	0.152	0.20
		SRA only	2	0	99.33	0.053	0.067	0.053	0.068	0.106	0.135	0.095	0.191	
	Reducing sugars	SRA and QSL	4	0	0.17	0.026	0.064	14.944	36.754	0.052	0.127	0.064	0.127	
		SRA and QSL B	4	0	0.22	0.026	0.029	11.651	13.187	0.052	0.059	0.029	0.059	0.10
		SRA only	2	0	0.22	0.022	0.031	9.828	13.763	0.044	0.061	0.043	0.087	
	Starch	SRA and QSL	4	0	48.70	1.959	3.203	4.023	6.578	3.918	6.407	3.203	6.407	
		SRA and QSL B	4	0	47.39	1.959	2.822	4.134	5.956	3.918	5.644	2.822	5.644	-2.62
		SRA only	2	0	47.39	1.471	3.258	3.104	6.876	2.941	6.516	4.608	9.215	
	Finegrain	SRA and QSL	4	1	23.69	2.696	4.047	11.380	17.082	5.393	8.095	4.047	8.095	
		SRA and QSL B	4	1	25.07	2.696	3.852	10.756	15.366	5.393	7.704	3.852	7.704	2.65
		SRA only	2	1	24.79	1.314	2.562	5.300	10.336	2.628	5.124	3.623	7.246	

B: bias adjusted, N: number of contributors, Outl: number of outliers, RPBLTY: repeatability, RPDCBLT: reproducibility, SD: standard deviation, Uncert.: uncertainty

Table 19: Precision metrics for raw sugar population experiment

		Ash	Colour	Dextran	Filtr.	Fines	GGM	Moist.	Moist. F	Pol	Pol LP	Pol F	RS	Starch
Reproducibility	Reproducibility SD (S_R)	0.003	33.979	0.562	0.594	0.245	54.681	0.005	0.004	0.016	0.016	0.020	0.007	0.298
	Reproducibility (R)	0.006	67.959	1.124	1.187	0.490	109.363	0.010	0.009	0.031	0.033	0.039	0.014	0.595
	Standard uncertainty (u)	0.003	33.979	0.562	0.594	0.245	54.681	0.005	0.004	0.016	0.016	0.020	0.007	0.298
	Expanded uncertainty (U)	0.006	67.959	1.124	1.187	0.490	109.363	0.010	0.009	0.031	0.033	0.039	0.014	0.595
SRA reproducibility	Repeatability SD (S_r)	0.007	52.046	2.655	1.179	0.544	45.119	0.005	0.016	0.024	0.031	0.033	0.015	0.585
	Repeatability (r)	0.013	104.092	5.309	2.357	1.087	90.238	0.010	0.031	0.049	0.062	0.065	0.030	1.171
Tully Mill reproducibility	Repeatability SD (S_r)	0.009	27.424	1.091	1.156	0.401	12.792	0.006	0.010	0.016	0.026	0.035	0.013	0.361
	Repeatability (r)	0.018	54.848	2.182	2.311	0.803	25.585	0.013	0.021	0.031	0.053	0.070	0.026	0.723

Filtr.: Filtrability, Moist.: Moisture, F: Fresh raw sugar, LP: low purity, RS: reducing sugars

Table 20: Published precision data for Australian and international wet chemistry methods for sugarcane factory products (2001a, Bartens, 2011)

Method	Matrix	Analytes	Expected range	Repeatability	Reproducibility
Method 2 – Pol: Determination in juice[1]	Juice	Pol % juice	0 - 30 %	0.10	Not determined
Method 3 – Brix: Determination in juice by hydrometer[1]	Juice	Brix % juice	12 - 30 °Bx	0.20	Not determined
Method 3A – Brix: Determination in juice by vibrating tube density measurement[1]	Juice	Brix % juice	12 - 30 °Bx	0.08	Not determined
Method 4 – Fibre: Determination in cane[1]	Prepared cane	Fibre % cane	8 - 20 %	0.25	Not determined
Method 4A – Fibre: Determination in cane by SRA can fibre machine[1]	Prepared cane	Fibre % cane	8 - 20 %	0.29	Not determined
Method 5 – Sampling and analysis for evaluating milling train performance[1]	Prepared cane and bagasse	Brix % cane Pol % cane Moisture % cane Pol in open cells % Fibre % cane by disintegrator Fibre % cane by bag		0.18 0.40 0.76 3.2 0.49 0.86	Not determined
GS5/7-1 – Determination of pol, brix and fibre in cane and bagasse by wet disintegrator[2]	Cane Bagasse	Brix % cane Pol % cane Moisture % cane Fibre % cane		Not determined	Not determined
GS1/2/3/9-1 – Polarisation[2]	Raw sugar	Pol °Z		0.10	0.25
GS5/1 – Reducing sugars in cane raw sugar by the Luff Schoorl procedure[2]	Raw sugar	Reducing sugars %	For sugars containing 0.18 - 0.74 %	0.02	0.05
GS1/3-7 – Solution colour at pH 7.0[2]	Raw sugar	Colour (ICUMSA)	500 - 2000	110	380
GS1/3//7/8-13 – Conductivity ash[2]	Raw sugar Juice Syrup Molasses	Ash	Raw sugar average ash 0.4 %	0.028	0.084

Table 20: Published precision data for Australian and international wet chemistry methods for sugarcane factory products cont.

Method	Matrix	Analytes	Expected range	Repeatability	Reproducibility
GS1/2/3/9-15 – Dextran by modified alcohol haze method[2]	Raw sugar	Dextran mg/kg		40	80
GS2/15 – Sugar moisture by loss on drying[2]	Raw sugar	Moisture		Not determined	Not determined
GS4/7-1 – Sucrose by double pol[2]	Molasses	Sucrose %		Not determined	Not determined
GS7/8/4-24 – SGF in pan products by HPIC[2]	Juice Syrup Molasses	Sucrose %	9 – 13 % 30 – 35 %	0.14 0.73	ND 1.24
GS4/3-3 – Total reducing sugars in mol[2]	Molasses	Reducing sugars %		0.77	1.60
GS4/7-11 – Dry substance and moisture in molasses by drying on sand[2]	Molasses Cane syrups	Dry substance %		0.84	2.77
GS7-7 – Pol in filter cake[2]	Mud	Pol %	1.0 – 5.0 %	Not determined	Not determined
GS7-9 – Moisture in filter cake[2]	Mud	Moisture %	70 – 80 %	Not determined	Not determined

[1] ICUMSA Methods book, [2]BSES Laboratory Methods for Australian sugar mills Volume 2 2001, ND: not determined

6.1.4.3. Specificity

Selectivity describes a method's ability to quantify the analyte in the presence of other material. Specificity describes a method that works only for the analyte and is not influenced by other products in the matrix, such as degradation that appear as analytes (Barton II, pers. comm.). It can be very difficult to test an analytical method for specificity. Typically, for an HPLC method, the sample is put under stress conditions to form degradation products. The sample is then analysed using multiple HPLC detectors online and the chromatograms compared. If they are different, it is assumed that the analyte is impure (Huber, 2010). The selectivity and specificity of NIR spectroscopic methods depend heavily on the specificity and selectivity of the reference methods upon which they are based. If the reference method is both specific and selective for an analyte, NIR spectroscopy typically works well, however if the analytical method is only specific for an analyte under a certain set of conditions, the NIR method will typically show bias and/or skew (Barton II, pers. comm.). As NIR spectroscopic methods analyse the analyte in the matrix, this can occasionally stabilise a measurement where the reference chemistry begins to fail. One example of this may be the measure of pol in low purity juice samples.

The specificity of the NIR spectroscopic technique cannot be evaluated in the same way as an analytical technique. Where pure standards of compositional materials can be obtained and they exist in a non-interacting matrix, such as for pharmaceutical materials, specificity can be evaluated by comparing the calibration coefficient plot with a spectrum of a pure standard of the analyte. They should be the same. Unfortunately, for agricultural samples this is not possible, as almost all materials exist in an interacting matrix.

Instead, for agricultural samples, the relationship between the values derived from the reference method and the NIR spectroscopic method must be used to evaluate the specificity of the method. If the validation samples show skew (a statistically significant deviation of m from 1 in the linear equation y= mx+c), or an unexplained bias (a statistically significant deviation of c from 0 in the linear equation y= mx+c) the calibration model is not well related to the analyte of interest as measured by the reference chemistry.

In the validation of the Global 17.1 models, lack of specificity can be seen in the dextran and starch models for raw sugar, represented as a significant skew Figure 72 and Figure 73. The reference method for dextran measures any material that precipitates in a 10% solution of ethanol, which includes dextran, but also may other carbohydrates. Additionally, dextran itself is a class of compounds with different molecular weights and chain lengths. While the reference method will capture all of these molecules in its result for dextran, NIR spectroscopy will be sensitive to each of the different molecular weights of dextran as individuals, and consequently, will not match the wet chemistry well.

For analytes such as ash, which do not have an NIR spectroscopic signal directly, they are 'observed' by NIR spectroscopy through interaction with the matrix. Essentially, the NIR spectrometer sees their influence, but is blind to the cause. In this scenario, the method is not specific to the analyte, but rather, specific to the repeatable influence of the analyte on the sample matrix. In the NIR spectroscopic community, this is considered acceptable if the model validates appropriately.

6.1.4.4. Sensitivity

Sensitivity is the ability of a method to respond reliably and measurably to changes in analyte concentration (Harris, 2007).

Often, sensitivity is defined by limits of detection, limits of quantitation and limits of reporting, which are related to the smallest measured quantity of analyte that is statistically significantly different from the measurement of the blank. Once again, the lack of blank samples in agriculture, as well as the bulk analysis technique makes this difficult to evaluate for NIR spectroscopy, although a least squares approximation can be calculated.

The sensitivity of an analytical method is defined by the slope (m) of the calibration curve (provided in each of the calibration plots). However, this value is not particularly useful for evaluating if two concentrations can be discriminated. For this application, the minimum difference that can be distinguished between two measures was calculated from the standard deviation of the slope and the acceptance criteria defined by a t-test (Massart, 1998, Harris, 2007). The sensitivity for each of the calibration models currently in use is provided in Table 21.

Table 21: Sensitivity of NIR spectroscopic prediction models at 95% confidence

Product	Constituent	Slope	Uncertainty at 95% confidence	Smallest distinguishable difference (d)
Molasses G17.1	Dry Substance % Molasses	0.96	0.011	0.031
	Ash % Molasses	0.82	0.040	0.125
	Reducing sugars % Molasses	0.88	0.042	0.083
	Sucrose % Molasses	0.98	0.016	0.021
	Brix % Molasses	0.98	0.015	0.007
	Pol % Molasses	0.98	0.020	0.017
	True purity	0.98	0.029	0.022
	Acetic acid % Molasses	0.89	0.349	0.251
	Lactic acid % Molasses	0.96	0.256	0.153
Raw sugar G16.1	Pol % Sugar	0.93	0.005	0.015
	Pol - low purity % Sugar	0.98	0.002	0.007
	Moisture % Sugar	0.90	0.008	0.022
	Ash % Sugar	0.84	0.012	0.036
	Colour (ICUMSA)	0.95	0.006	0.008
	Reducing Sugars % Sugar	0.98	0.004	0.010
	Fine Grain %	0.70	0.014	0.052
	Filtrability	0.76	0.013	0.044
	Starch (ppm)	0.10	0.009	0.235
	Filtrability	0.05	0.050	2.359
Juice and syrup G17.1	Brix % Juice/Syrup	1.00	0.000	0.001
	Pol % Juice/Syrup	1.00	0.001	0.002
	Pol Reading (°Z)	1.00	0.001	0.002
	Ash % Juice/Syrup	0.99	0.010	0.010
	Reducing Sugars % Juice/Syrup	0.92	0.039	0.017
	CCS	0.71	0.606	0.621
	Sucrose % Juice/Syrup	0.96	0.066	0.044
	Dry substance % Juice/Syrup	0.93	0.104	0.063
	True purity	0.63	0.114	0.093

Table 21: Sensitivity of NIR spectroscopic prediction models at 95% confidence cont.

Product	Constituent	Slope	Uncertainty at 95% confidence	Smallest distinguishable difference (d)
Massecuite and magma G17.1	Brix % Massecuite	0.99	0.005	0.014
	Pol % Massecuite	0.99	0.004	0.011
	Dry Substance % Massecuite	0.97	0.006	0.017
	Sucrose % Massecuite	0.95	0.008	0.021
	Ash % Massecuite	0.97	0.039	0.047
	Reducing Sugars % Massecuite	0.94	0.039	0.108
	Crystal % Massecuite	0.86	0.083	0.249
	Impurity to Water Ratio	0.98	0.008	0.020
	Water % Massecuite	0.99	0.007	0.018
	True purity	0.96	0.006	0.017
Bagasse G16.1	Pol % Bagasse- Primary mills	0.98	0.004	0.010
	Pol % Bagasse- Final mill	0.89	0.017	0.032
	Fibre % Bagasse	0.97	0.012	0.016
	Moisture % Bagasse	0.90	0.016	0.019
	Brix % bagasse	0.37	0.834	0.880
Mill mud G16.1	Moisture % Mud	0.89	0.020	0.060
	Pol % Mud	0.94	0.016	0.030
	Fibre % Mud	0.56	0.069	0.160
	Mud Solids % Mud	0.70	0.082	0.120
	Total Insolubles % Mud	0.78	0.085	0.042
Prepared cane G16.1	Brix % Juice	0.99	0.003	0.008
	Pol % Juice	0.98	0.005	0.009
	Fibre % Cane	0.86	0.019	0.028
	Brix % Cane	0.98	0.008	0.008
	Pol % Cane	0.98	0.011	0.004
	CCS % Cane	0.97	0.015	0.011
	Ash % Cane	0.79	0.044	0.035
	DryMatter % Cane	0.95	0.028	0.017
	Moisture % Cane	0.95	0.032	0.017
	Pol in Open Cells (POC)	0.88	0.047	0.025

The smallest distinguishable difference describes the smallest difference in concentration that the technique can measure with 95 % confidence. Almost all of the key metrics for each product have sensitivities less than 0.03, with most less than 0.02. This is lower than the error of the method and the expected reporting and action thresholds, indicating the technique is sensitive enough for routine use.

6.1.4.5. Linearity

Linearity describes how well the calibration curve follows a straight line. The square of the correlation coefficient (R^2) is the most common measure of linearity. The closer the R^2 value is to 1, the better the linear fit.

The R^2 values for the Global 16.1 and 17.1 models are provided in Table 12 and Table 15. Most show values greater than 0.97, indicating excellent linearity. Some models, such as starch and dextran in raw sugar have poor R^2 values due to high scatter of the data and significant skew.

6.1.4.6. Range

Range is defined as the region of the calibration curve over which linearity, accuracy and precision are all maintained within acceptable limits. For NIR spectroscopic techniques, the calibration models are only suitable for predicting samples that are similar to those used to build the calibration model.

Therefore, range does not constitute only the relationship between the reference value and the NIR predicted value (which should be interpolated from the calibration set), but also requires that the spectrum of the new samples resides within the spectral variation of the calibration library, in multi-dimensional space. A spectrum is considered 'normal' if it has a GH metric less than 3 and an nH metric lower than 0.8. The GH and nH metrics are discussed further in the affiliated industry report on benchtop NIR spectroscopic systems (Keeffe and Staunton, 2017).

6.2. Sample characterisation

This section describes the chemical characterisation of key factory products and subsequent calibration model development, based on this information. The intended deliverables of this research activity were (a) a full chemical understanding of sugar factory products, (b) a suite of molecularly-targeted NIR calibrations for sugar factory products, and (c) an understanding of the curing process of fresh raw sugar and a strategy for its management when using spectroscopy methods for constituent concentration prediction.

6.2.1. Characterisation of nominated products

A selection of samples for each of the nine products of interest have been subjected to additional testing to understand their chemical composition. Priority was given to raw sugar, bagasse and molasses, as these have been identified as products of significance during mill trials. Depending on the analyte matrix, the samples were subjected to a range of testing, including: ion chromatography (IC), inductively coupled plasma- atomic emission spectroscopy (ICP-AES), thermogravimetric analysis (TGA) and Fourier transform (FT) infrared (IR) spectroscopy, among other things.

The samples were selected based on their origin and the distribution of the key analyte concentrations for each product. In essence, the extreme samples from a single mill were chosen to demonstrate as much variance in the analytes as possible, while maintaining consistency of product. This will assist in observing the spectral changes resulting from the changing sample matrix.

The characterisation and interpretation will be described for each product separately.

6.2.1.1. Molasses

Twenty five molasses samples were selected based on their origin and sugar/ solids composition. Initially, three A Molasses, B Molasses and C Molasses samples were selected from a mill that undertakes true purity measurements and three from a mill that undertakes apparent purity measurements. The three samples within each grade of molasses were also selected based on a distribution (high, medium, low) of analyte concentration. An additional six samples were selected due to unusually high or low values for a particular analyte. A summary of the samples can be seen in Table 22. Each sample was subjected to further analysis for moisture, dry solids, ash, sugars, major

and trace nutrient elements, and carbon. A selection of samples were also analysed by TGA, IR spectroscopy and Raman spectroscopy.

Table 22: Summary of molasses samples selected for characterisation

Sample name	Sample Type	Mill	Brix %	Pol °Z	Apparent purity %	Sucrose %	Dry subs. %	True purity %	Reducing sugars %	Ash %
AMol 0307	A Mol	Mill 9	74.88	58.72	78.4					
AMol 0345	A Mol	Mill 9	74.24	57.52	77.5					
AMol 0302	A Mol	Mill 9	73.84	56.12	76.0					
AMol 0284	A Mol	Mill 3				54.9	74.4	73.8		
AMol 0107	A Mol	Mill 3				53.9	76.3	70.6		
AMol 0101	A Mol	Mill 3				55	75.3	73.0		
AMol 0035	A Mol	Mill 7				48.5	69.8	69.5		
AMol 0056	A Mol	Mill 10	80.56	50.58	62.8					
AMol 0026	A Mol	Mill 11				58.3	79.4	73.4		
BMol 0164	B Mol	Mill 9	69.23	48.52	70.1					
BMol 0326	B Mol	Mill 9	73.48	50.44	68.6					
BMol 0153	B Mol	Mill 9	78.44	52.16	66.5					
BMol 0289	B Mol	Mill 3				45.8	73.6	62.2		
BMol 0072	B Mol	Mill 3				46.8	72.5	64.6		
BMol 0082	B Mol	Mill 3					71.2			
BSyr 0079	B Mol	Mill 3				55.5	75.4	73.6		
BMol 0085	B Mol	Mill 6				49.5	73.4	67.4		
CMol 0336	C Mol	Mill 9	80.52	32.87	40.8	34.4	79.4	43.3	14.6	13.36
CMol 0319	C Mol	Mill 9	84.64	38.85	45.9					
CMol 0333	C Mol	Mill 9	83.44	36.56	43.8					
CMol 0275	C Mol	Mill 3	75.96			33.8	77.8	43.4		
CMol 0114	C Mol	Mill 3	86.5			33.4	76.5	43.7		
CMol 0073	C Mol	Mill 3	86.1			32	71.8	44.6		
CMol 533	C Mol	Mill 6				30.5	71.3	42.8		
CMol 0008	C Mol	Mill 11	86.2			38.1	80.2	47.5		

The two mills operated at different purities during the period the samples were taken. In both cases, the purity dropped from A to C molasses, as expected. Both mills showed overlap in the wet chemistry results between A and B molasses for brix. For example, Mill 9 had brix values of 73.84 % - 74.88 % for A molasses and 69.23 % - 78.44 % for B molasses. The variance in the associated pol value, however, maintained the apparent purity for A molasses (76.0 % - 78.42 %) and B molasses (66.5 % - 70.0 %) in the expected ranges.

Each of the samples selected for analysis contained missing data due to individual mills' analytical procedures, comparing analytic responses is difficult. To supplement the mill laboratory wet chemistry, the NIR predicted values for each of the constituents were predicted from the Global 16.1 calibration model, which is the most up-to-date at the time of this experiment. These values are provided in Table 23, along with calculated purity values. Mostly, the correlation between the wet chemistry values and the predicted values were very strong, however, there were a few samples with high residuals, marked in grey.

Table 23: Predicted NIR spectroscopic values for molasses samples

Sample name	Sample Type	Mill	Brix %	Pol °Z	Apparent purity %	Sucrose %	Dry subs. %	True purity %	Reducing sugars %	Ash %
AMol 0307	A Mol	Mill 9	75.4	62.4	82.8	62.2	71.3	87.2	17.0	5.6
AMol 0345	A Mol	Mill 9	73.5	57.2	77.9	50.9	68.2	74.6	19.4	6.2
AMol 0302	A Mol	Mill 9	73.2	61.5	83.9	64.4	69.7	92.4	15.3	6.3
AMol 0284	A Mol	Mill 3	78.7	55.8	70.9	55.4	74.1	74.7	18.5	8.3
AMol 0107	A Mol	Mill 3	81.5	55.6	68.3	54.8	77.2	71.0	17.6	9.1
AMol 0101	A Mol	Mill 3	81.3	54.4	66.9	55.9	76.9	72.7	17.0	9.4
AMol 0035	A Mol	Mill 7	75.4	47.8	63.4	52.3	70.2	74.4	14.3	8.1
AMol 0056	A Mol	Mill 10	81.6	55.2	67.6	51.1	76.2	67.1	19.9	7.3
AMol 0026	A Mol	Mill 11	83.3	60.9	73.1	61.6	79.6	77.4	14.6	9.5
BMol 0164	B Mol	Mill 9	70.1	37.7	53.8	64.8	67.4	96.1	4.5	11.7
BMol 0326	B Mol	Mill 9	74.1	50.2	67.7	55.9	69.5	80.4	13.4	7.3
BMol 0153	B Mol	Mill 9	74.5	49.9	67.0	49.4	68.5	72.1	15.4	7.9
BMol 0289	B Mol	Mill 3	79.0	47.9	60.6	45.6	73.2	62.2	16.5	8.3
BMol 0072	B Mol	Mill 3	79.1	46.3	58.5	46.6	72.3	64.4	14.6	7.7
BMol 0082	B Mol	Mill 3	76.9	47.0	61.1	46.2	70.6	65.3	15.1	8.4
BSyr 0079	B Mol	Mill 3	80.7	60.1	74.5	59.0	76.6	77.0	16.0	8.4
BMol 0085	B Mol	Mill 6	85.7	55.0	64.2	56.5	80.3	70.4	12.7	10.3
CMol 0336	C Mol	Mill 9	80.4	33.8	42.1	36.7	72.2	50.8	10.5	11.2
CMol 0319	C Mol	Mill 9	83.4	37.7	45.2	40.2	74.5	54.0	9.2	13.1
CMol 0333	C Mol	Mill 9	82.8	35.9	43.4	38.6	73.8	52.3	9.0	12.8
CMol 0275	C Mol	Mill 3	87.0	30.0	34.5	34.1	77.9	43.8	12.0	13.2
CMol 0114	C Mol	Mill 3	86.1	28.7	33.4	33.0	76.8	43.0	11.7	12.9
CMol 0073	C Mol	Mill 3	81.1	26.3	32.4	28.8	72.1	40.0	12.1	10.6
CMol 533	C Mol	Mill 6								
CMol 0008	C Mol	Mill 11	86.8	35.8	41.2	38.2	79.0	48.3	15.5	11.7

Obtaining the moisture and dry solids values for molasses was extremely difficult due to the very low mass transfer of the product, likely due to its high salt and sugar loadings. Attempts to oven dry the samples at various temperatures and under vacuum failed due to the formation of a crust on the surface of the sample, which prevented moisture loss from the sample below. Eventually the surface of the sample would degrade and the sample was no longer viable. Analysis under vacuum would cause the sample skin to swell and overflow the sample container.

After considerable experimentation, a method was developed to analyse the samples for moisture and dry solids by freeze-drying. The moisture and dry solids values for each sample are provided in Table 24.

The moisture values ranged from 14.9 % - 34.9 %, with the majority of samples between 22 % and 29 %. Consequently, the dry solids were relatively stable at between 75 % - 82 %. There was not a strong relationship between change in dry solids and molasses grade. There was a linear relationship between the dry solids by freeze drying and NIR prediction, however it was offset by 3 % - 4 %. This is similar to the relationship between the dry solids by freeze drying and dry substance values obtained at the mill.

Table 24: Freeze-drying results for molasses samples

Sample name	Sample Type	Mill	Moisture %	Dry solids %
AMol 0307	A Mol	Mill 9	24.5	75.5
AMol 0345	A Mol	Mill 9	25.7	74.3
AMol 0302	A Mol	Mill 9	27.1	72.9
AMol 0284	A Mol	Mill 3	21.3	78.7
AMol 0107	A Mol	Mill 3	19.6	80.4
AMol 0101	A Mol	Mill 3	20.1	79.9
AMol 0035	A Mol	Mill 7	24.4	75.6
AMol 0056	A Mol	Mill 10	21.6	78.4
AMol 0026	A Mol	Mill 11	17.4	82.6
BMol 0164	B Mol	Mill 9	34.9	65.1
BMol 0326	B Mol	Mill 9	27.7	72.3
BMol 0153	B Mol	Mill 9	26.3	73.7
BMol 0289	B Mol	Mill 3	22.9	77.1
BMol 0072	B Mol	Mill 3	21.8	78.2
BMol 0082	B Mol	Mill 3	22.9	77.1
BSyr 0079	B Mol	Mill 3	29.9	70.1
BMol 0085	B Mol	Mill 6	15.4	84.6
CMol 0336	C Mol	Mill 9	24.4	75.6
CMol 0319	C Mol	Mill 9	20.5	79.5
CMol 0333	C Mol	Mill 9	22.6	77.4
CMol 0275	C Mol	Mill 3	14.9	85.1
CMol 0114	C Mol	Mill 3	18.1	81.9
CMol 0073	C Mol	Mill 3	21.1	78.9
CMol 533	C Mol	Mill 6	24.7	75.3
CMol 0008	C Mol	Mill 11	15.1	84.9

The sugars in the samples were quantified by IC. An example of the molasses chromatogram is provided in Figure 94.

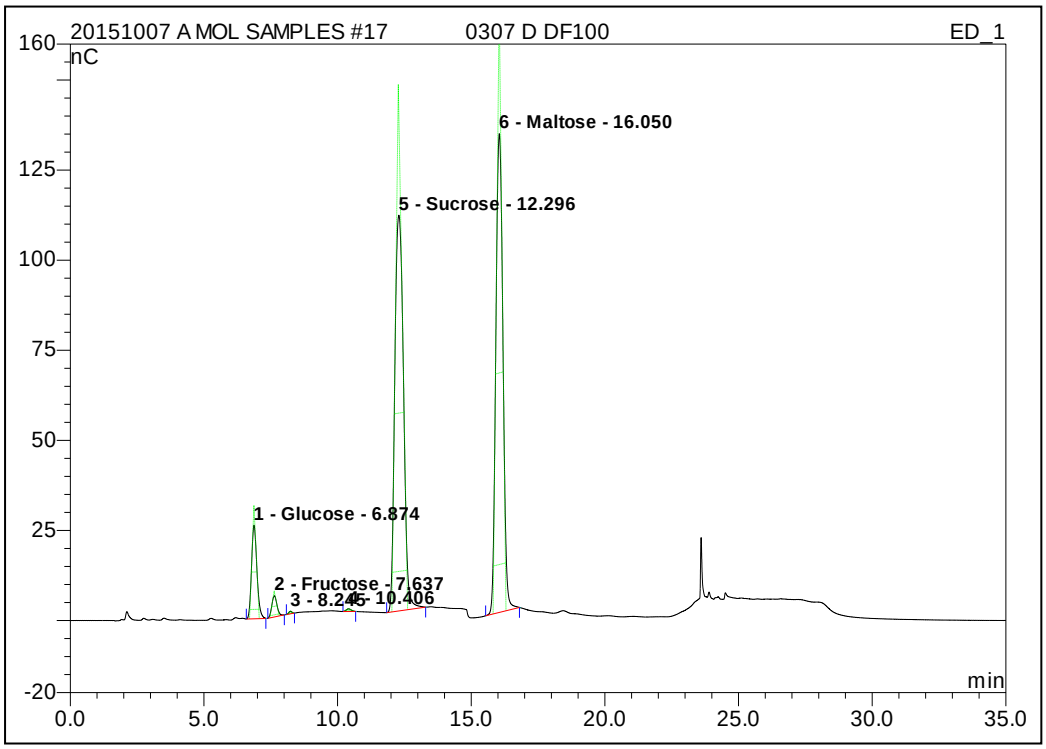

Figure 94: Molasses chromatogram showing sucrose, glucose, fructose and maltose (internal standard)

The chromatogram shows strong signals from sucrose, glucose, fructose and maltose, which was used as an internal standard. Peaks were well separated and the baseline showed few other trace-level carbohydrates. The concentration of the three sugars for each sample is shown in Table 25. As expected, sucrose was the most abundant sugar in each sample and declined from A to C Molasses. As was observed in the mill-wet chemistry, samples from Mill 9 were typically higher in sucrose. The relationship between the sucrose by IC and the mill-wet chemistry was strong and linear. The IC and NIR predicted results showed a similar relationship, but there was slightly more scatter about the regression line, particularly at higher concentration. Glucose and fructose concentrations were typically under 5 % and increased from A to C molasses. Ion chromatography glucose and fructose values were much lower than the predicted values for reducing sugars and showed no relationship. The NIR predicted results were comparable with the single value provided by the mill-wet chemistry.

Table 25: Ion chromatography results for molasses samples

Sample name	Sample Type	Mill	Glucose %	Fructose %	Sucrose %
AMol 0307	A Mol	Mill 9	1.6	1.3	56.0
AMol 0345	A Mol	Mill 9	1.6	1.2	51.7
AMol 0302	A Mol	Mill 9	1.8	1.4	52.8
AMol 0284	A Mol	Mill 3	3.4	2.4	51.5
AMol 0107	A Mol	Mill 3	4.6	3.3	49.3
AMol 0101	A Mol	Mill 3	3.4	2.9	52.4
AMol 0035	A Mol	Mill 7	3.6	3.3	45.1
AMol 0056	A Mol	Mill 10	5.6	4.6	48.1
AMol 0026	A Mol	Mill 11	3.0	2.9	56.2
BMol 0164	B Mol	Mill 9	2.0	1.1	43.0
BMol 0326	B Mol	Mill 9	2.5	1.9	48.3
BMol 0153	B Mol	Mill 9	2.5	1.9	51.4
BMol 0289	B Mol	Mill 3	5.2	4.1	45.7
BMol 0072	B Mol	Mill 3	4.0	3.2	44.2
BMol 0082	B Mol	Mill 3	3.6	2.8	46.8
BSyr 0079	B Mol	Mill 3	0.2	0.2	46.0
BMol 0085	B Mol	Mill 6	5.1	4.9	50.7
CMol 0336	C Mol	Mill 9	3.5	3.9	33.3
CMol 0319	C Mol	Mill 9	3.4	3.9	38.0
CMol 0333	C Mol	Mill 9	4.0	4.6	36.4
CMol 0275	C Mol	Mill 3	7.0	0.5	32.8
CMol 0114	C Mol	Mill 3	5.3	5.2	32.1
CMol 0073	C Mol	Mill 3	4.6	4.6	32.1
CMol 533	C Mol	Mill 6	7.4	7.4	30.4
CMol 0008	C Mol	Mill 11	5.5	6.0	37.4

Major and trace elements were measured by ICP-AES. Samples were dissolved in water prior to analysis. Carbon and nitrogen were measured by the DUMAS combustion method. The values for both techniques are shown in Table 26. All values showed an increase from A to C molasses, which is expected due to the concentrating effect that removal of sucrose has. The calcium values are very high across the board, due to the lime addition during clarification. The samples from Mill 3 are considerably higher in sodium than the other mills for all molasses grades. The A molasses samples from Mill 7, Mill 11 and Mill 10 have very high iron and manganese levels, compared to the other A molasses samples. The Mill 6 B molasses sample *B Mol 0085* showed many results that were either higher or lower than the other B molasses samples and often showed a similar nutrient profile to C molasses.

Table 26: ICP-AES results for molasses samples

Sample name	Sample Type	Mill	Ca mg/L	Fe mg/L	K mg/L	Mg mg/L	Mn mg/L	Na mg/L	P mg/L	S mg/L	C %	N %
AMol 0307	A Mol	Mill 9	3774	16.11	11750	2421	16.62	231.7	203.5	2971	28.4	0.31
AMol 0345	A Mol	Mill 9	4090	20.22	12080	2205	18.18	204.5	229	3161	28.5	0.34
AMol 0302	A Mol	Mill 9	3623	12.8	13000	2296	14.85	194.4	217.8	3151	28.1	0.33
AMol 0284	A Mol	Mill 3	3278	15.24	6640	1879	8.184	521.5	139.5	2138	30.5	0.42
AMol 0107	A Mol	Mill 3	3966	30.71	11220	3381	17.25	953.9	235.5	2938	30.9	0.35
AMol 0101	A Mol	Mill 3	4399	19.61	9916	2889	11.74	768.8	197.6	3003	30.7	0.40
AMol 0035	A Mol	Mill 7	3679	48.83	12600	2478	18.9	548.4	353.2	2479	28.2	0.31
AMol 0056	A Mol	Mill 10	4172	38.01	13230	1960	34.73	124.9		1615	30.8	0.38
AMol 0026	A Mol	Mill 11	4173	61.29	15720	1207	28.12	197.3	389.1	1450	31.6	0.40
BMol 0164	B Mol	Mill 9	3175	20.63	14850	2673	29.51	300.5	240.1	2517	25.3	0.34
BMol 0326	B Mol	Mill 9	5147	20.05	16310	3033	30.92	345.8	277.2	4227	27.3	0.41
BMol 0153	B Mol	Mill 9	4741	30.27	16100	3221	29.29	398.9	294.5	3789	27.9	0.43
BMol 0289	B Mol	Mill 3	5385	36.08	14940	4233	24.66	1666	306.1	4645	29.3	0.44
BMol 0072	B Mol	Mill 3	5250	28.54	15490	4492	14.3	1084	327.3	4081	28.9	0.47
BMol 0082	B Mol	Mill 3	5521	39.05	14170	4115	12.36	1345	272.4	3612	28.5	0.47
BSyr 0079	B Mol	Mill 3	3077	49.48	13110	1954	14.36	834.3	207.1	2832	26.8	0.24
BMol 0085	B Mol	Mill 6	5344	49.16	20260	1902	41.66	177.5	415.2	2379	32.6	0.42
CMol 0336	C Mol	Mill 9	7968	47.76	35660	5721	40.93	572.1	487.4	6433	26.5	0.84
CMol 0319	C Mol	Mill 9	9352	40.18	32050	6304	40.72	664.9	504.8	7205	27.2	0.79
CMol 0333	C Mol	Mill 9	8242	38.73	32760	6406	48.54	590.1	513.4	7102	27.7	0.82
CMol 0275	C Mol	Mill 3	9928	50.79	26160	7636	23.79	1674	486.8	6650	30.1	0.82
CMol 0114	C Mol	Mill 3	9161	48.56	26080	7529	24.51	2056	589.5	6865	29.6	0.75
CMol 0073	C Mol	Mill 3	10088	50.44	25400	6872	22.83	1580	468.8	5792	27.9	0.74
CMol 533	C Mol	Mill 6	6039	81.32	26380	4294	36.16	877.2	460.4	6762	27.4	0.54
CMol 0008	C Mol	Mill 11	7692	86.14	29920	2584	84.62	821	644.2	2627	31.1	0.57

A principal component analysis (PCA) was conducted on the wet chemistry characterisation data (mill wet chemistry, NIR predicted wet chemistry and analytical wet chemistry) to observe the correlations that exist between the techniques. All data was autoscaled prior to analysis to give a mean of zero and standard deviation of one. This allows comparison of variance between the variables measured in different units. A biplot produced during the analysis is provided in Figure 95 and a marked up version describing the following relationships is provided in Figure 96. The NIR predicted variable names for the loadings are preceded by a *P*, e.g. Brix by mill laboratory is labelled *Brix*, and brix predicted by NIR spectroscopy is labelled *PBrix*.

The biplot shows that 54 % of the variance in the data is described by principal component (PC) 1 and 19 % of the variance is explained by PC2. The gross relationship identified on PC1 is the change in purity from A molasses to C molasses, with distinct scores groups identifiable for each A molasses, B molasses and C molasses (black groups in Figure 96). Within these clusters, there is a spread of data along PC2, which describes the moisture/solids relationship, with some discrimination between solids from salts, and solids from sugars and other organic matter (OOM). The clusters for A and B molasses are overlapping partially and both *BSYR0079* and *AMol0035* are clustering with opposite groups. This overlap is due to the overlap in target of A molasses and B molasses across the factories (Table 27) and was also reflected in the raw values as described above.

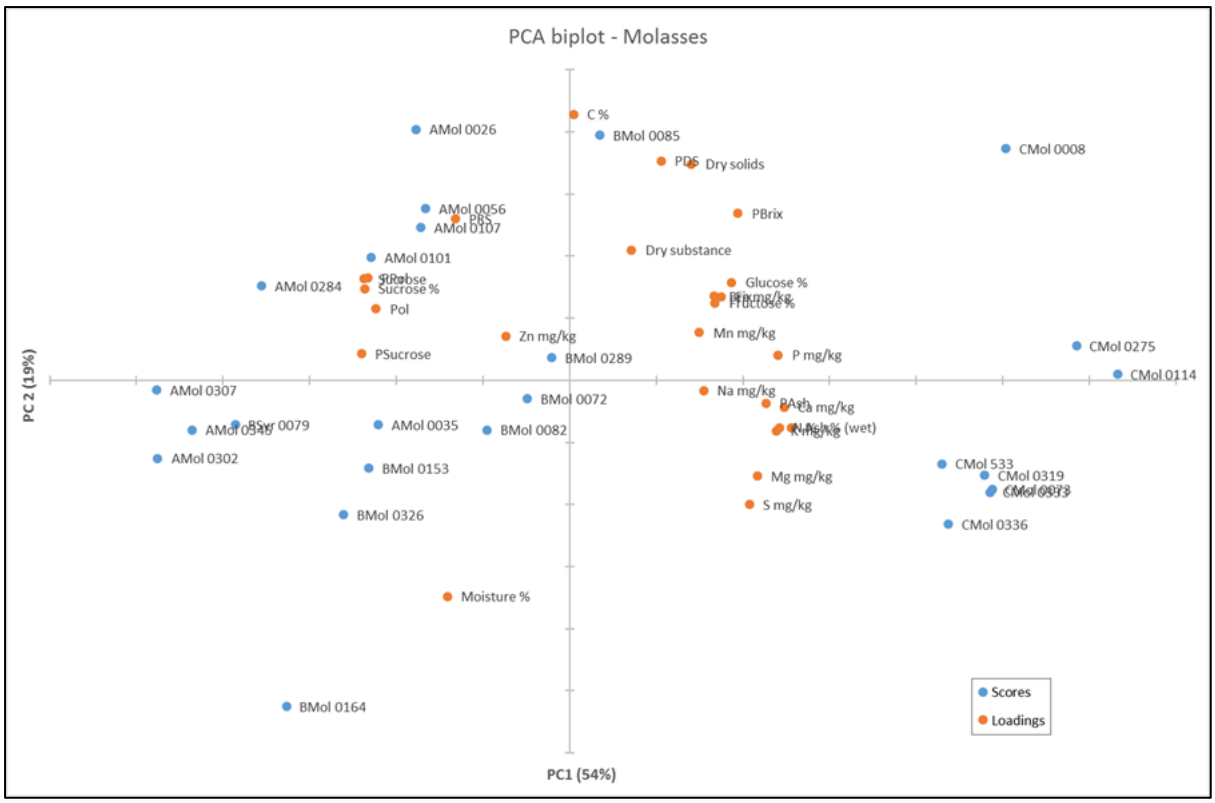

Figure 95: PCA biplot of all mill wet chemistry, NIR predicted wet chemistry and analytical wet chemistry for molasses samples

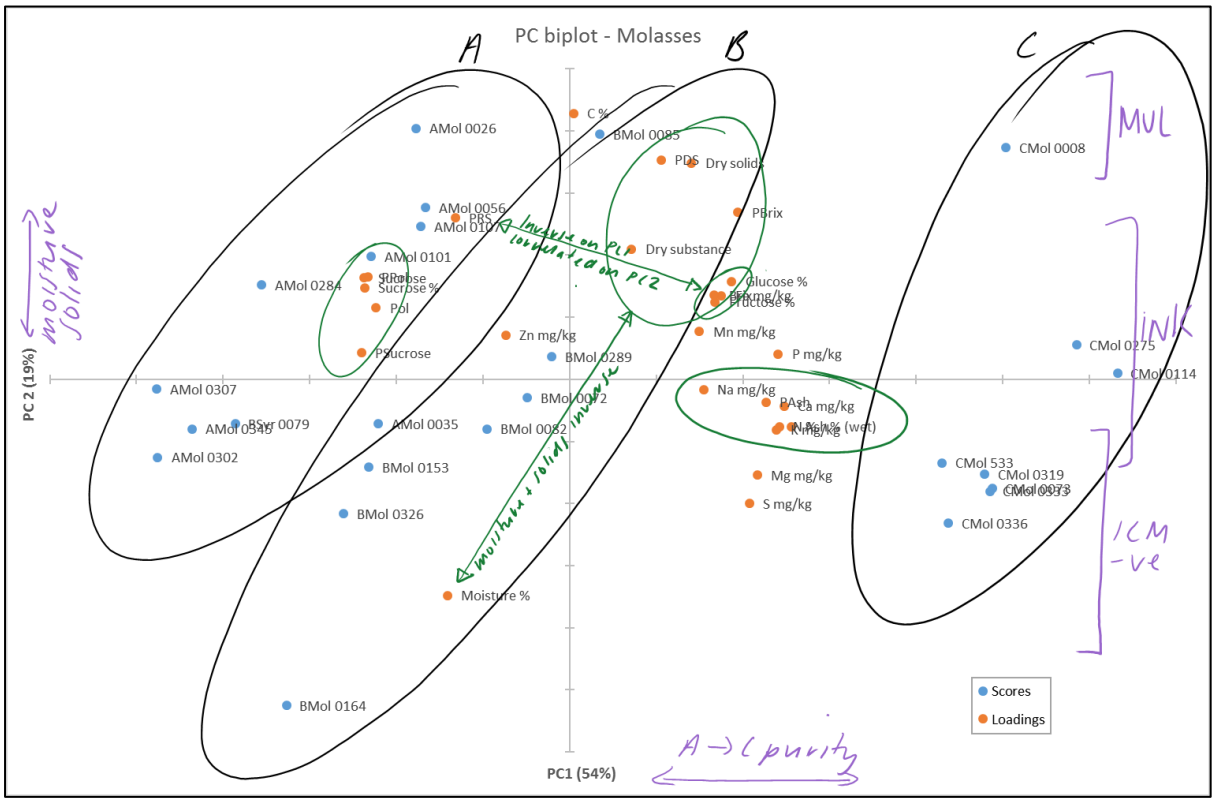

Figure 96: PCA biplot of molasses samples showing mark-up

Table 27: Factory targets for mill products

Sample name	Sucrose	Dry substance	Water	Purity
Liquor	67 - 62	66 - 69	32 - 35	87 - 93
A Molasses	52 - 57	70 - 74	27 - 32	71 - 75
B Molasses	45 - 57	72 - 76	28 - 32	64 - 69
C Molasses	31 - 37	75 - 79	21 - 25	40 - 47
A Massecuite	79 - 84	89 - 93	7 -11	86 - 91
B Massecuite	75 - 78	89 - 93	7 -11	80 - 86
C Massecuite	60 - 65	89 - 93	7 -11	65 - 69
Magma	76 - 80	88/89	8 - 12	88/89

The distribution of the loadings shows that the three sources of wet chemistry data provided similar results for each parameter or type of parameter. This can be seen by the clustering of *sucrose*, *pol*, *ppol* and *psucrose*; *PDS*, *dry solids*, *pbrix*, *brix* and *dry substance*; and, *pash*, *ash%* and *salts (Na, K, Ca)*. *Moisture* was inverse to the solids, as would be expected. One interesting difference, which was also reflected in the raw wet chemistry values, was the difference between the *glucose* and *fructose* values and *PRS*. Their positioning on the biplot at right angles to each other indicates no relationship. While both positive on PC2, they are opposite on PC1 and *PRS* correlates more with the sucrose measurements. This may indicate two things: first, the NIR spectroscopic model for *PRS* may not be specific to reducing sugars; instead, measuring total sugars or sucrose; or, glucose and fructose are not the only reducing sugars in the sample identified by the mill wet chemistry methods (on which the NIR models are based).

A second PCA was conducted that included a second derivative, standard normal variate (SNV) NIR spectrum with the wet chemistry. This illustrates the change in the NIR spectroscopic response with the change in purity of the sample and allows the active regions of the spectrum to be identified. Combining it with the wet chemistry allows relationships between specific spectral regions and the wet chemistry to be evaluated. The PCA biplot is provided in Figure 97. The distribution of the objects and variables in this biplot is slightly different to that in Figure 96 due to the influence of the NIR spectra. However, there is still separation of the three grades of molasses in response to a purity gradient as seen previously. The biplot is complemented by a plot of the second derivative SNV NIR spectra compared to the loadings plots for PC1 and PC2 (Figure 98). The loadings plots presented in this way facilitate an evaluation of which regions of the spectrum are best contributing to the model. The information captured in the biplot in Figure 97 has been annotated on the spectrum/loadings plot in Figure 99 for easy interpretation.

The NIR spectra showed strong chemical variance throughout the spectrum that was typically observed in shifting absorbance at specific wavelengths. Often, this shifting showed separation between the A and C molasses samples, but there was overlap between the A and B molasses and the B and C molasses. Some regions of the spectrum showed wavelength shifting between samples, particularly in the 7250 - 6900 cm^{-1} region. This is most likely due to the hydrogen bonding being affected by the presence of salts in the samples (Workman and Weyer, 2008, Molt et al., 1998, Lin and Brown, 1994, Hirschfield).

Some samples show a very strong absorbance at 6963 cm^{-1}, which is non-specific to molasses grade. Further investigation identified a strong absorption for crystalline sucrose in this region, which is illustrated in Figure 100.

Crystalline sugar has very little moisture and consequently shows little activity in the 7300 – 6900 cm^{-1} region, except for this peak, which has been assigned with two potential vibrations: the first overtone of intermolecularly H-bonded O-H stretch (2000), and/or -CH_2 C-H combination (Shenk et al., 2001). Davies and Miller (Davies and Miller, 1988) indicate that the sharp band at 6944 cm^{-1} is characteristic of crystalline sucrose and is ascribed to a non-hydrogen-bonded form of OH group that is "frozen" into the crystal structure (Miller, 2001). This indicates that the samples that show activity in this region of the spectrum may have formed small sucrose crystals during transport and storage.

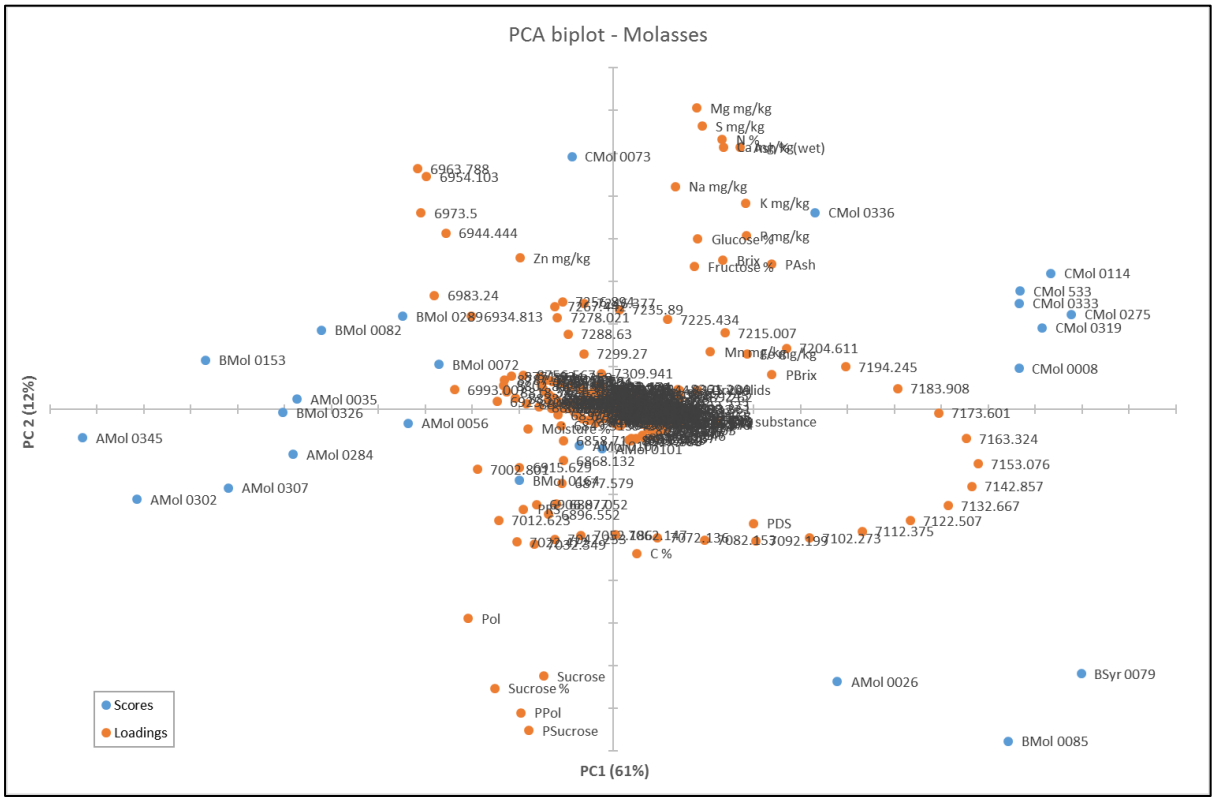

Figure 97: PCA biplot of molasses samples NIR spectra and wet chemistry

In Figure 99, the major peaks (observed as troughs in 2SNV NIR spectra) have had chemical vibrations assigned based on literature values. Most assignments relate to water stretching and bending vibration modes (Iwamoto et al., 1986, 2000), as well as CH, CH_2 and CH_3 vibrations (2000, Murray and Williams, 1987), which likely arise from the sugars in the solution. There are possible assignments between 8900 - 8800 cm^{-1} and 7092 cm^{-1} that represent aromatic and olefinic vibrations, which may be present as Maillard by-products, polyphenolics or flavonoids (Poel et al., 1998).

The biplot shows that there is strong correlation between the C molasses and the 7200 - 7100 cm^{-1} region, which also correlated with dry substance, solids and brix. This is inverse to A and B molasses. The strong PC1 loading at this wavelength suggests it is a significant contributor to the identification of non-sucrose dry solids in the sample. The NIR spectra in this region show a clear wavelength shift. An additional region contributing to dry solids and brix, and consequently separation of C molasses, is 8389 - 8347 cm^{-1}, which is also positive on PC1.

The biplot in Figure 97 shows that the major cause of separation on PC2 is due to the presence of sucrose (negative on PC2) or the presence of salts (ash) and reducing sugars (positive on PC2).

These variables are influencing the separation of A and B molasses. The CH, second overtone region of the spectrum between 7062 cm^{-1} and 6850 cm^{-1} is correlated with high pol and sucrose samples and inversely correlated with ash and brix. There is a strong positive in the PC2 loadings at 6963 cm^{-1}, which is ascribed to a B molasses response, countering the rest of the region's strong A molasses relationships. B molasses is also distinguished from A molasses through the active regions at 8818 cm^{-1} and 7610 cm^{-1}.

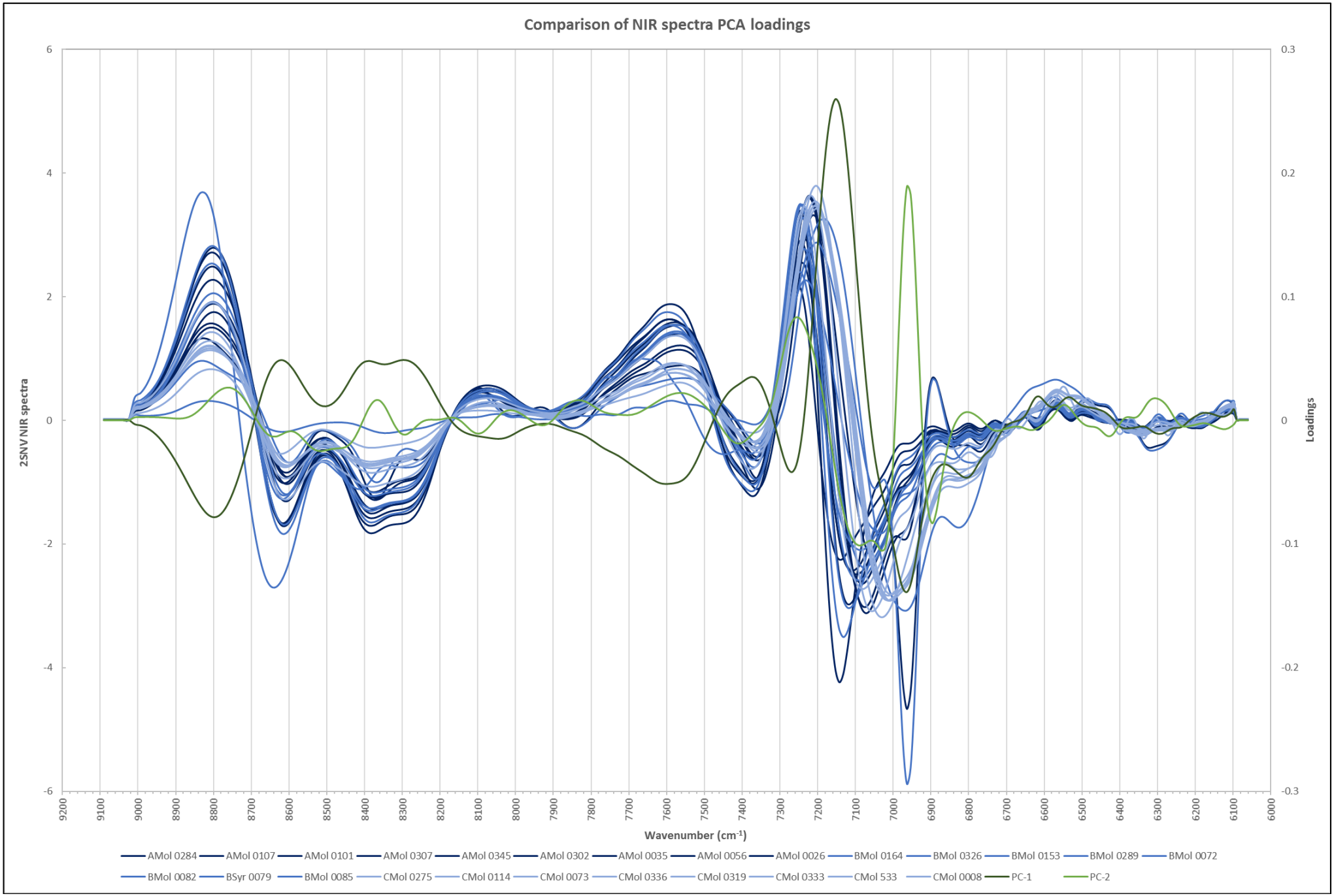

Figure 98: Spectra of molasses samples compared to PCA loadings

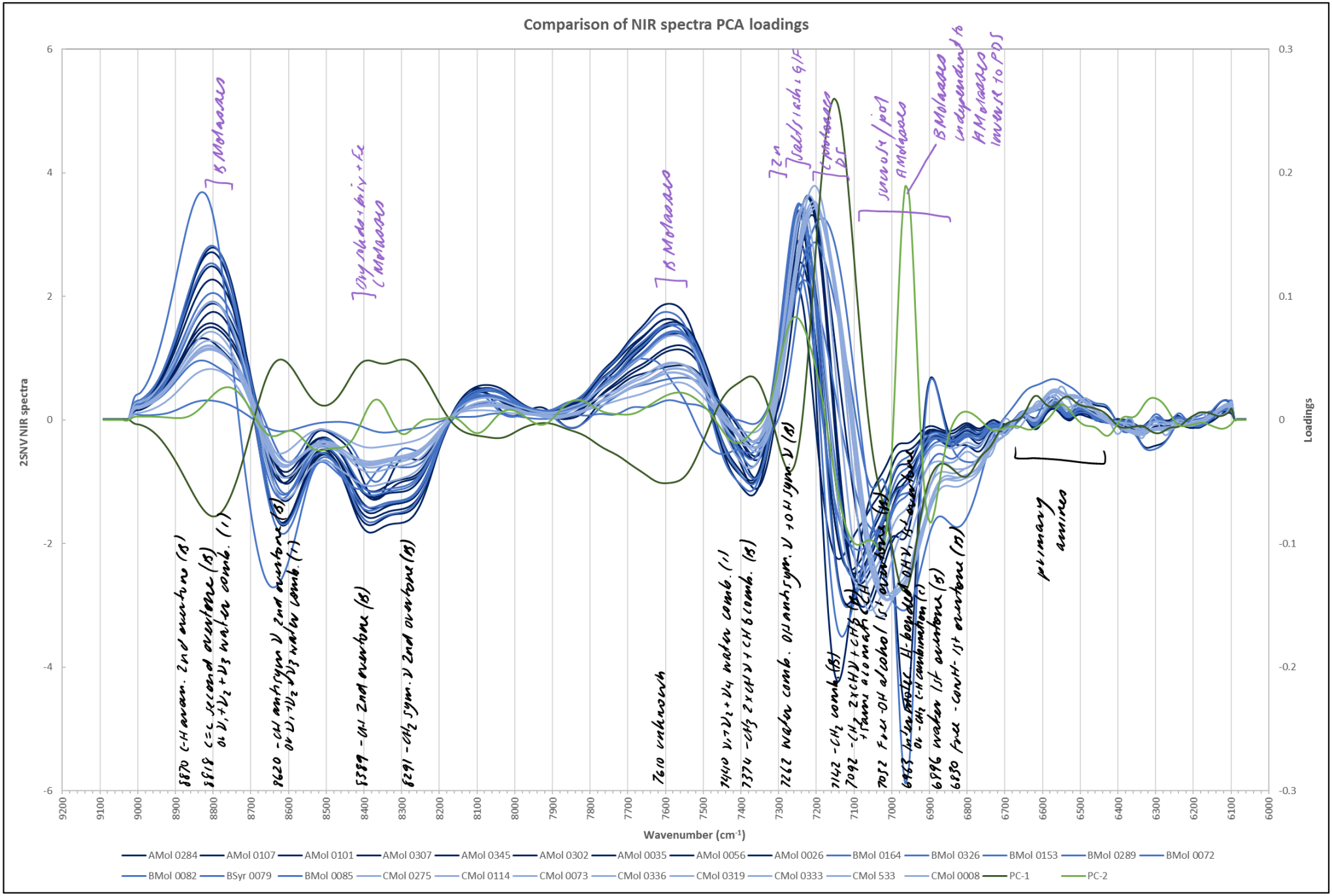

Figure 99: Spectra of molasses samples compared to PCA loadings showing mark-up (2000)

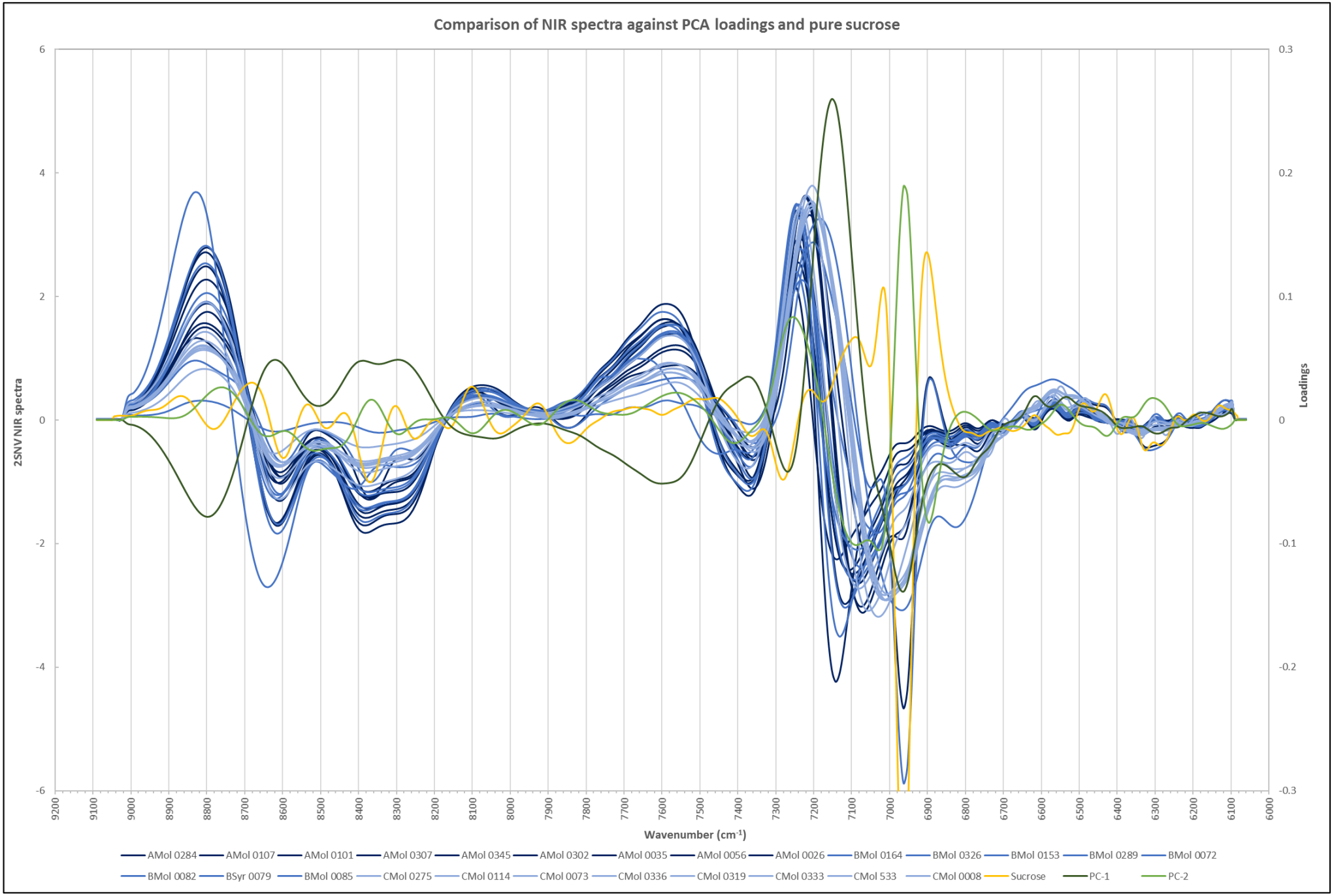

Figure 100: Comparison between molasses NIR spectra, PCA loadings and pure sucrose spectrum

Additional analyses were conducted to further characterise the samples in an attempt to identify and other unusual characteristics that would facilitate the NIR spectral interpretation and subsequent calibration development. The nine samples collected from Mill 9 were selected for further characterisation.

These samples were subjected to Raman spectroscopy, FT-IR spectroscopy and TGA. The Raman spectroscopic technique failed to collect suitable spectra as the fluorescence from the organic components was masking the Raman signal. Attempts were made to minimise the fluorescence by quenching the sample, testing multiple laser wavelengths.

Infrared spectra were collected using a Nicolet 5700 FT-IR fitted with a diamond ATR accessory. The spectra were subjected to ATR correction (Diamond – angle 45, bounce 1, RI 1.50) and normalised using the SNV algorithm. The spectra are illustrated in Figure 101 and the following observations are annotated on the spectra in Figure 102.

The FT-IR spectra of molasses show strong water vibrations, which appear as broad peaks and mask many of the sharp prescriptive peaks expected of IR spectroscopy. In particular are the large H-bonded OH stretch vibration at 3332 cm^{-1} and the OH bending vibration at c. 1600 cm^{-1}. Despite this, some interpretation of the spectra was feasible.

The spectra of the nine molasses samples were very consistent, with the only variations appearing to be chemical in nature, as expected. Typically, the variations that existed trended with the purity of the sample. The C molasses samples showed the largest differences, particularly in the 1650 – 1300 cm^{-1} region, where the absorbance was much stronger. Another interesting feature to note is the slight shift in frequency of the water peak at 3332 cm^{-1}. It is well understood that the bending and stretching vibrations of compounds shift to higher and lower frequencies, respectively, upon the addition of water (Williams and Fleming, 1995). This is due to the increase in hydrogen bonding and weakening of covalent bonding. In this case, the C molasses samples are at a lower frequency than the B molasses samples, which are lower than the A molasses samples. This is the opposite of what was expected as an increase in the salts of a system, should increase the disruption to the hydrogen bonding and consequently increase the frequency of the vibration (Molt et al., 1998, Lin and Brown, 1994, Hirschfield, Keeffe, 2013). The cause of this inversion is unknown, but may be a result of the hydrogen bonding availability of the sugars in the molasses solution.

The 1800 - 400 cm^{-1} region of the IR spectrum is known as the fingerprint region and is often used to identify pure compounds. This region is particularly sensitive to the structural features of various carbohydrates, including sugars. Previous work (Robert et al., 1993) has identified key peaks for sucrose, glucose and fructose in this region and these have been marked in green in Figure 102. The most significant peaks in this region at 1135, 1106, 1049, 993 and 925 cm^{-1} are all ascribed to sucrose vibrations. Shoulders and minor peaks can also be seen at glucose (1146, 1106, 1074, 1050, 1030 and 996 cm^{-1}) and fructose (1154, 1100, 1070, 1036, 1020 cm^{-1}) vibrations.

Thermogravimetric analyses were conducted on all of the selected molasses samples and two samples (*AMol 0302* and *AMol 0307*), were also analysed by TGA-MS. TA Instruments Q500 TGA instruments were used. Samples were analysed using a platinum pan, with nitrogen as the balance and sample gas. The temperature program had a 10 minute isothermal period followed by a ramp at 5 °C/ min to 1000 °C.

Figure 103 shows the mass loss spectra generated from the TGA analyses, as well as the derivative mass loss, standardised by SNV. The derivatised spectra show that the thermal degradation follows a similar process for each of the samples; however, differences occur at around 150 °C and 800 °C.

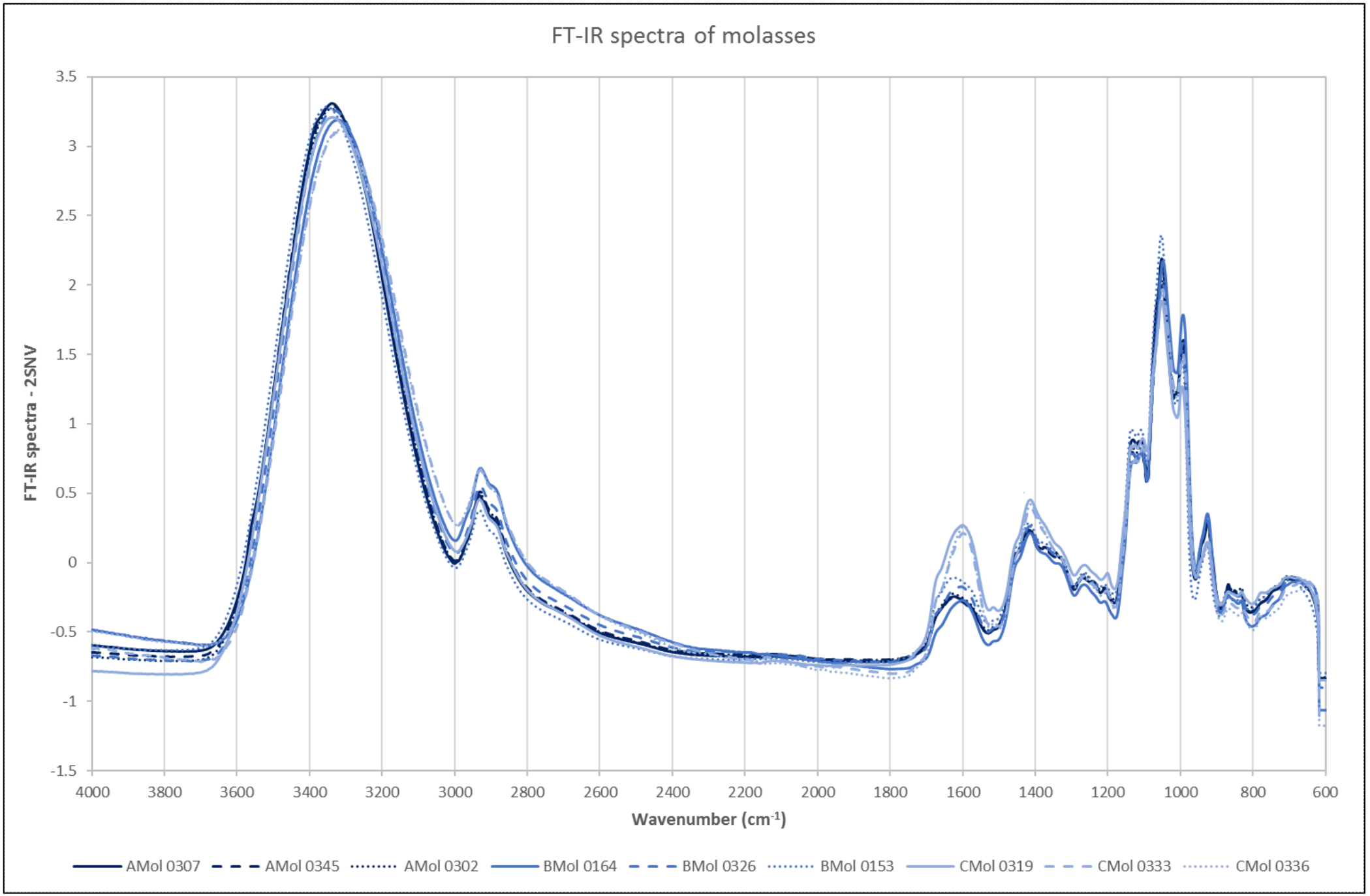

Figure 101: FT-IR spectra of selected molasses samples

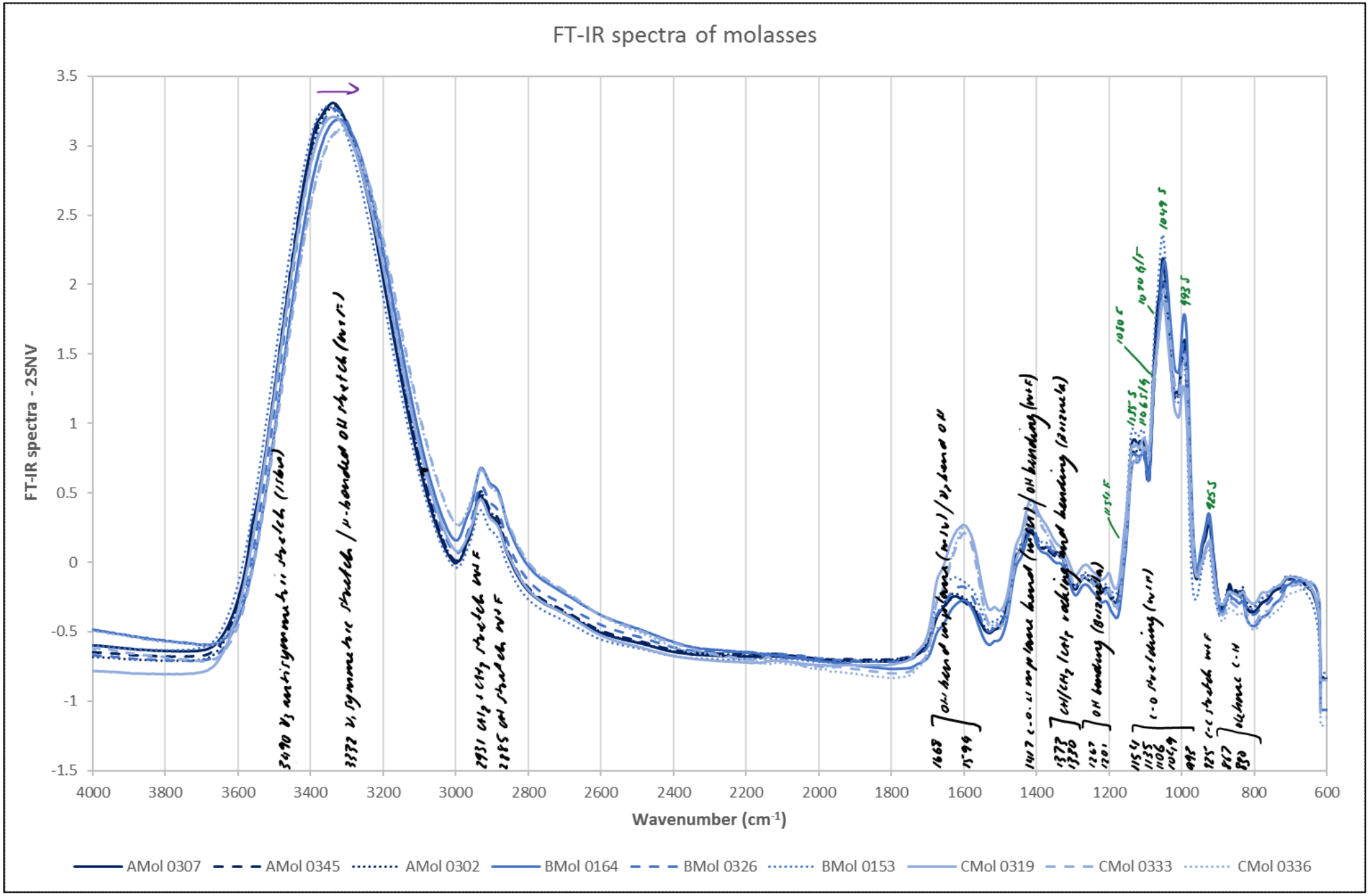

Figure 102: FT-IR spectra of selected molasses samples showing mark-up (Reusch, 2013)

There is also a slight temperature shift for the peak at 203 °C, which corresponds with an increase in purity. Figure 104 shows the derivative mass loss spectra, annotated with information obtained from the TGA-MS spectra collected from the A molasses samples. Much like during the freeze drying process, TGA analysis of the molasses samples was difficult due to swelling of the sample. Where the swelling contacted the thermocouple the analysis was repeated with a lower sample mass.

The heating of molasses initiates a series of extremely complex reactions about which, little specifics are known. In particular are the caramelisation and Maillard reactions. Caramelisation reactions are caused by the degradation of sugar in the presence of water, whereas Maillard reactions occur between reducing sugars and amino acids or other amines.

The first observable mass loss for molasses is at 83 °C and is attributed to water. Likely this is simple evaporation of water from the surface of the molasses, but may also be due to the Amadori rearrangement of glucose and the Heyns rearrangement of fructose, which are initial reaction pathways for Maillard reactions (Poel et al., 1998). During this time, it is also likely that mutarotation of sucrose is occurring, which is the preliminary stage of caramelisation reactions. This would not cause mass loss of the sample. The region between 100 - 175 °C shows considerable variability between the samples. This region is attributed to a loss of water and may represent the condensation and fragmentation reactions occurring in the Maillard process to produce dicarbonyl compounds. Alternatively, this loss of moisture may also be attributable to the β-elimination and retro-aldolisation reactions of the caramelisation process (Poel et al., 1998).

The large peak at 200 °C represents a rapid and significant mass loss of around 20 %. The TGA-MS identified a large number of compounds generated during this periods that are indicative Maillard (including Strecker) and caramelisation reactions (Poel et al., 1998, Mlotkiewicz, 2005). These include: furan (A_r 68) furfural (A_r 96), pyruvaldehyde (A_r 72), pyrrole (A_r 67), pyridine (A_r 79), imidazole (A_r 68), diacetyl (A_r 86), dimethyl furan (A_r 96), formic acid (A_r 46) and carbon dioxide (A_r 44). Interestingly, there was an absence of hydroxymethylfurfural (A_r 126), lactic acid (A_r 90) and ethyl acetate (A_r 88), which are known intermediates of these reactions. Similar products were observed up to 400 °C.

The carbon dioxide produced during the Strecker degradation is the cause for foaming of molasses and massecuites and may be a contributing factor in the expansion of molasses on freeze-drying and heating.

The variation between molasses classes in this region is likely to be caused by the presence of salts in differing ratios and concentrations. Potassium and calcium are known to catalyse the alkaline degradation of D-fructose (Poel et al., 1998). Similarly, the presence of salts catalyse the hydrolysis of sucrose to varying degrees (Poel et al., 1998), which is the rate limiting reaction for some of the degradation pathways. Another cause of variation between temperatures for the different samples may be the fructose content, which is much higher in the C molasses samples. Fructose caramelises at 110 °C, whereas glucose and sucrose caramelise at 160 °C.

Finally, another region of difference between the brands of molasses is the region between 650 and 850 °C, where the C molasses samples show a mass loss that the A and B molasses samples do not replicate. The cause of this is unknown at this point as the TGA-MS was collected only on A molasses samples. However, it is proposed that these components are ash-related due to the very high boiling point.

To compare the information collected across all analysis techniques, PCA was used once again.

The variables of the PCA comprised the mill wet chemistry, NIR predicted wet chemistry and analytical wet chemistry, which was autoscaled; NIR spectral data, which was treated with 2nd derivative SNV; FT-IR spectral data, which was ATR-corrected then SNV treated; and the TGA derivative mass loss data, which was treated with SNV. Only the nine Mill 9 samples were included. The combined spectra used in the analysis, overlaid with the first two loadings of the PCA are provided in

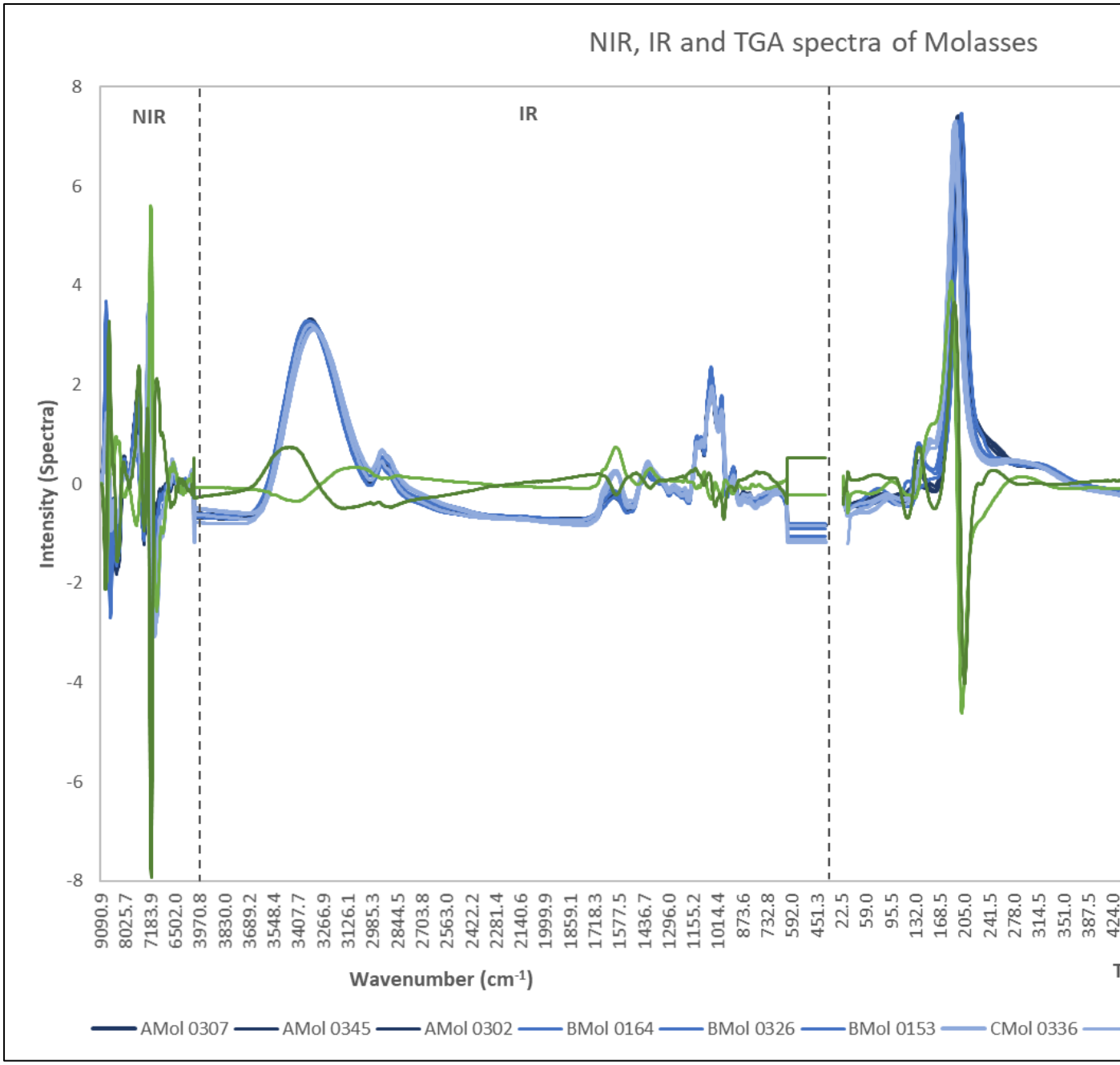

Figure 105. The associated PCA biplot is provided in Figure 106 and the biplot annotated with the following discussion is provided in Figure 107. For clarity, only relevant loadings have been labelled.

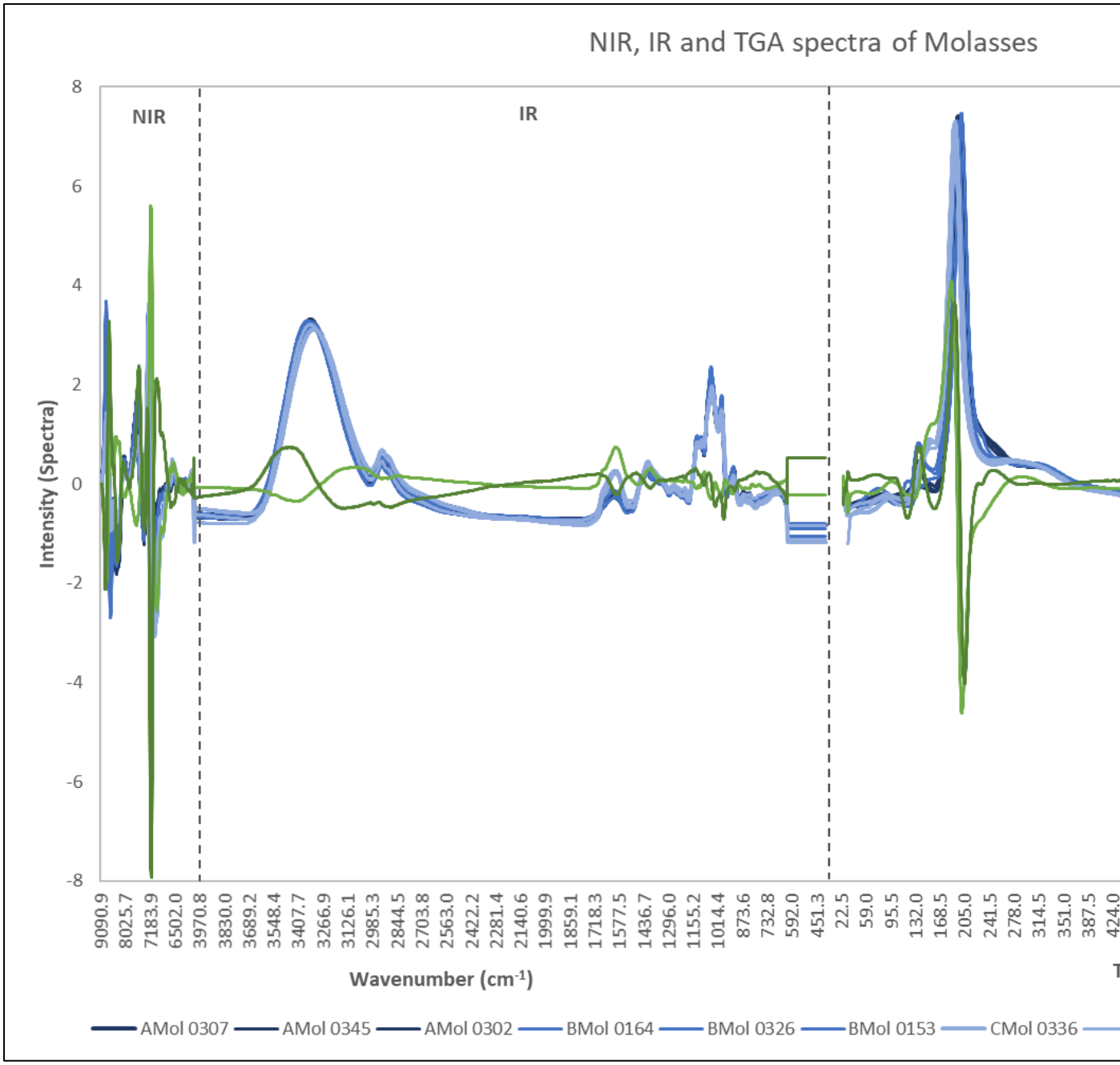

Figure 105 shows the variables contributing most to the models are in the TGA and NIR spectra. In particular, they are the 100 - 240 °C and 620 - 800 °C regions of the TGA and the 9000 - 8300 cm^{-1} and the 7700 - 6800 cm^{-1} regions of the NIR spectra. This is represented alongside the wet chemistry in Figure 106 and Figure 107.

The distribution of the scores in this biplot is similar to that seen in Figure 96, with change in purity the largest influence on the distribution of PC1 and the moisture/solids relationship observed in the PC2 distribution. The variables for each of the key descriptive groups (pol/sucrose, ash, solids, moisture) have maintained their clusters and relationships relative to each other, however, there is slightly more spread in each of the clusters and an overlap between solids and ash.

The maintenance of a similar structure with the addition of two new analytical techniques is a good indication that each of the techniques are identifying real chemical differences between each of the

samples. The three forms of spectral data account for 4,140 additional variables, which would override the chemistry variables if they were incompatible.

The correlations and relationships demonstrated in the biplot support the interpretation for each technique are provided earlier and give an indication of key variables to target in calibration development. There is a correlation between the pol/ sucrose parameters and NIR frequency 6993 cm^{-1}, which is assigned to the intermolecularly H-bonded OH stretch. In Figure 96 this band is correlated with sucrose on PC1, but inverse to sucrose on PC2, which identified it as a discriminating band for B molasses. The Mill 9 samples do not have a strong absorbance in this region, unlike the selection of samples in the larger population that were suspected to contain crystalline sucrose. This is likely to be the cause of difference and should be observed closely during calibration development.

The ash components correlated with the water combination and OH antisymmetric stretch at 7225 cm^{-1} and CH_2 combination band at 7160 cm^{-1}. These were related in Figure 96 as well and demonstrate the impact of salts on the frequency of the moisture vibrations, as well as the non-sucrose organic matter (OOM).

It was proposed that the salt loading in the samples caused the variance about the 200 °C peak in the TGA analyses. This is supported by Figure 107, which shows a strong shift in temperature with change in solids/ salts and sucrose/moisture that is equally distributed across PC1 and PC2.
The lower purity the sample, the lower the temperature of decomposition.

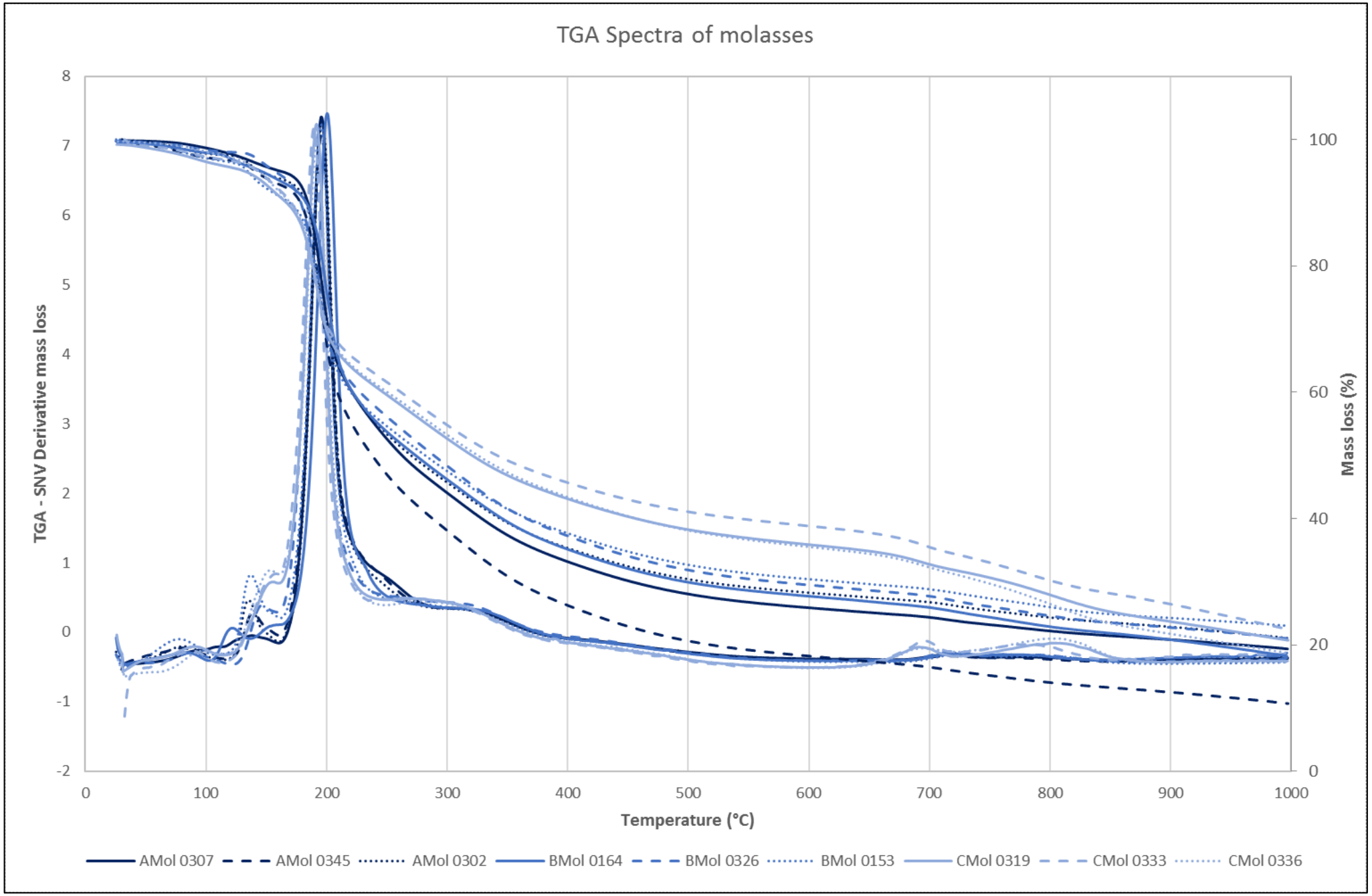

Figure 103: TGA SNV derivative mass loss spectra of selected molasses samples

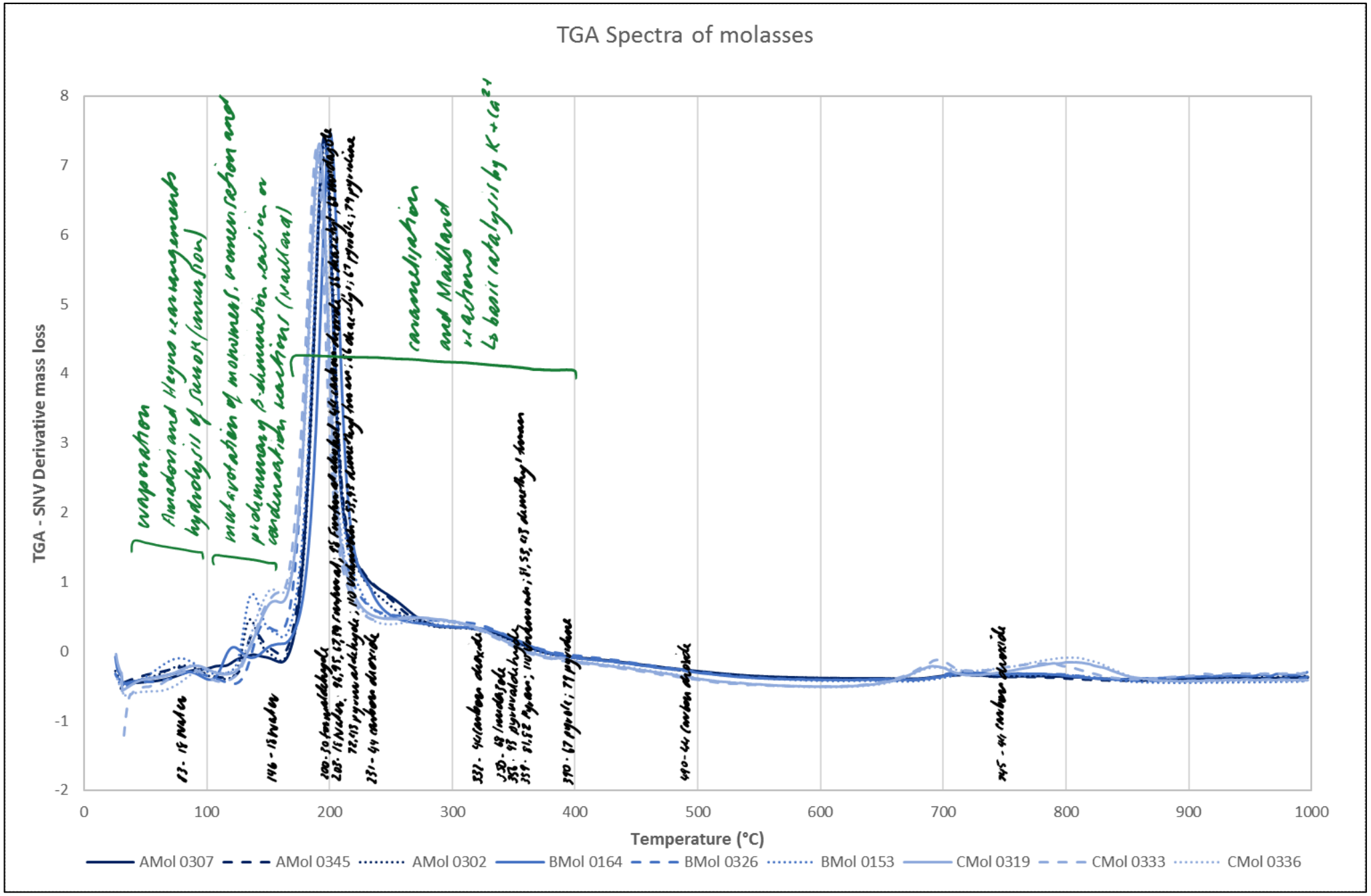

Figure 104: TGA SNV derivative mass loss spectra of selected molasses samples showing markup (Poel et al., 1998)

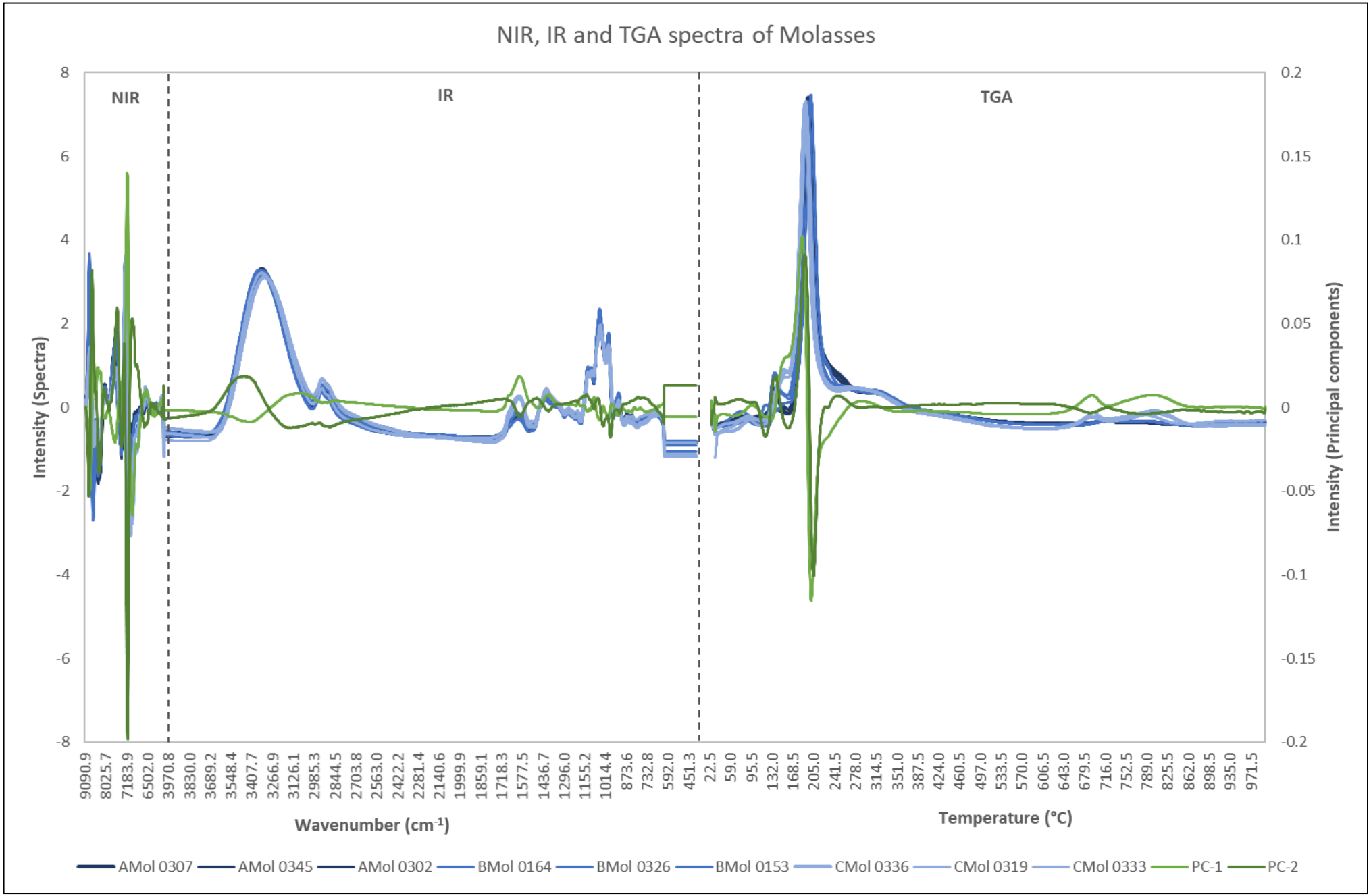

Figure 105: NIR, IR and TGA spectra of molasses samples showing loadings for PC1 and PC2

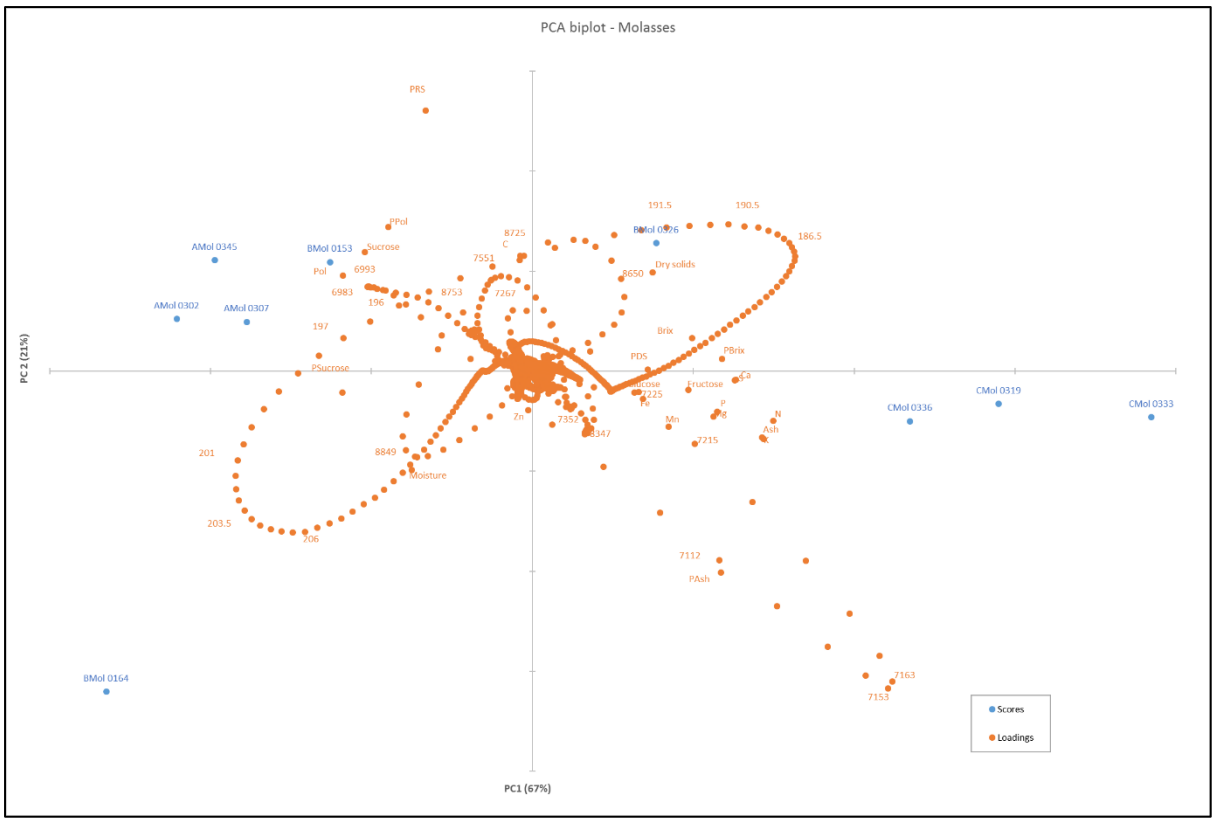

Figure 106: PCA biplot of molasses samples (WC, NIR, IR, TGA)

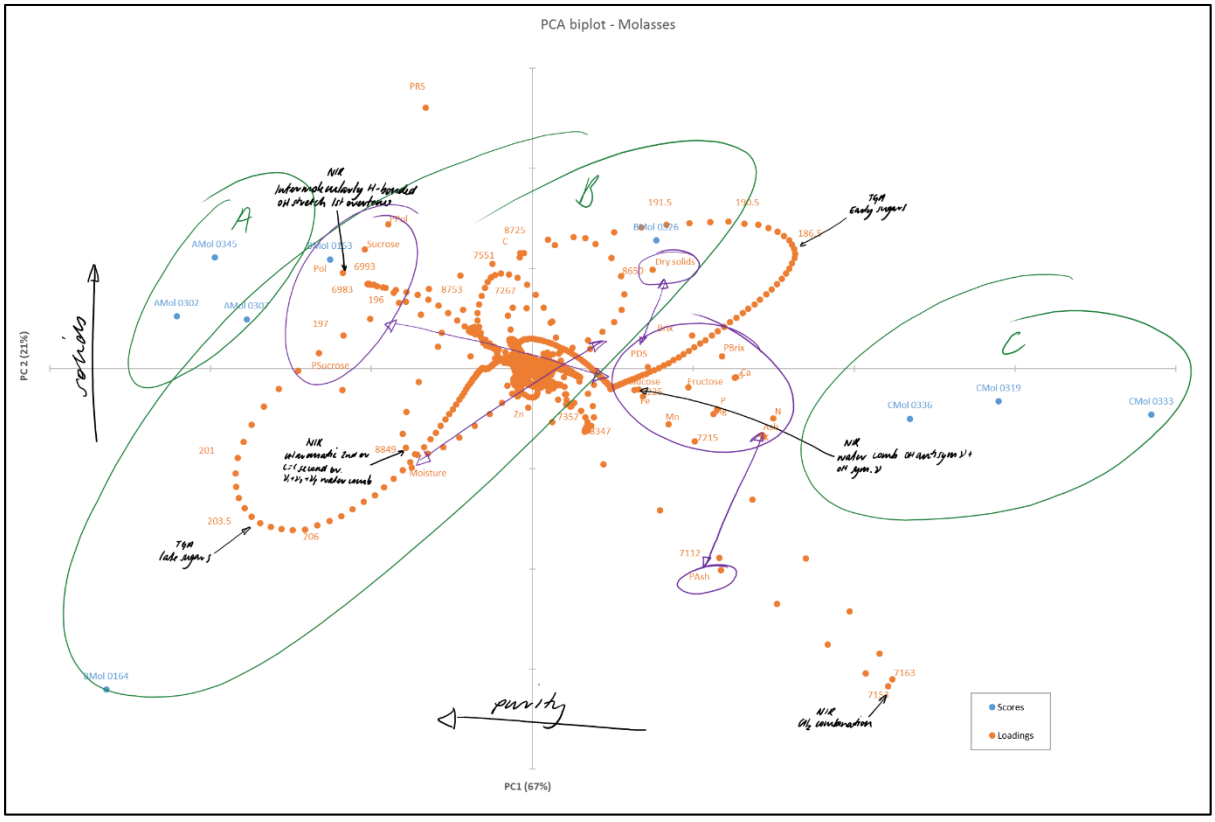

Figure 107: PCA biplot of molasses samples (WC, NIR, IR, TGA) marked up

The analysis described in this section has identified specific spectral regions that can be correlated with the wet chemistry data. These correlations are both positive and negative and may form a basis for developing molecularly-targeted calibration models. Specific vibrations of interest for each analyte are described in Table 28. The FOSS instrument uses wavelength as the unit for the spectral data, while this section has used wavenumbers for easy comparison between the NIR and FT-IR data. The regions were identified in the wavenumber domain and converted to a similar wavelength range. A lookup table for conversion between wavenumber and wavelength is provided in Appendix 2.

Table 28: Active NIR spectroscopic regions by analyte

	Wavenumber (cm^{-1})	Wavelength (nm)	Correlation
Sucrose/pol	7300-7200	1370-1390	Negative
	7100-7000	1405-1430	Positive
	6900-6800	1450-1470	Positive
Dry substance/brix	8850-8750	1130-1145	Negative
	8660-8580	1155-1165	Positive
	8400-8250	1190-1215	Positive
	7215-7082	1385-1415	Positive
	7000-6900	1425-1450	Negative
Ash/reducing sugars	8400-8330	1190-1200	Positive
	7250-7200	1380-1390	Positive
	7050-7000	1415-1430	Negative
	6900-6850	1450-1460	Negative
	6570-6520	1520-1535	Positive

Note: the remaining products will not be subjected to the same level of analyses based on the outcomes in Section 6.2.2. It is recommended that this section be read prior to reading the remainder of Section 6.2.1.

6.2.1.2. Raw sugar

Three raw sugar samples were collected from Mill 7. The three samples varied considerably in their pol and moisture values, which are summarised in Table 29. The moisture ranges from a very low 0.08 % to 0.21 % and the pol values varied inversely to the moisture, decreasing from 99.75 to 99.31 °Z. As with molasses, the samples were subjected to a prediction by the Global 16.1 calibration suite and the predicted values for the full set of sugar analytes is provided in Table 30. Overall, there was a good relationship between the NIR predicted values and the mill wet chemistry values. Some variance was observed, however, for sample *RS0027*. As would be expected, the colour and ash dropped with increasing pol. Samples were re-analysed for moisture and ash by single sulfation (2001a) at the Indooroopilly laboratory and the results are provided in Table 31. The values trended similarly to the NIR predicted and mill wet chemistry values, but the moisture values were slightly lower and the ash values were almost half of those predicted by NIR spectroscopy. The ash results may reflect an inexperienced operator as the method defines an expected concentration range for raw sugar to be between 0.20 - 0.60 % (2001a). The analytical wet chemistry ash values will not be used further. The calibration models for fine grain, filterability, starch and dextran have very high errors and their use for this purpose is not suitable.

Table 29: Summary of raw sugar samples selected for characterisation

Sample name	Sample Type	Mill	Moisture %	Pol °Z
RS 0152	Raw sugar	Mill 7	74.88	58.72
RS 0040	Raw sugar	Mill 7	74.24	57.52
RS 0027	Raw sugar	Mill 7	73.84	56.12

Table 30: Predicted NIR spectroscopic values for raw sugar samples

Sample name	Sample Type	Mill	Moist. %	Pol °Z	Colour (IU)	Ash %	RS %	Dextran %	Filtr. %	Fines %	Starch %
RS 0152	Raw sugar	Mill 7	0.18	99.30	0.18	99.29	1200	0.24	0.11	65.72	72.73
RS 0040	Raw sugar	Mill 7	0.08	99.75	0.08	99.65	618	0.18	0.00	81.59	82.18
RS 0027	Raw sugar	Mill 7	0.26	99.00	0.21	99.31	1504	0.27	0.07	94.21	69.93

Table 31: Moisture and ash results for raw sugar samples

Sample name	Sample Type	Mill	Moisture %	Ash %
RS 0152	Raw sugar	Mill 7	0.16	0.13
RS 0040	Raw sugar	Mill 7	0.07	0.09
RS 0027	Raw sugar	Mill 7	0.18	0.18

The sugars in the sample were quantified by IC in the same way as the molasses samples. The results are provided in Table 32. As expected the sucrose values increased with increasing pol. The reducing sugars were much higher in sample *RS 0152*, which was also reflected in the predicted RS value. Although the sample with the second highest predicted RS had the lowest glucose and fructose values.

Table 32: Ion chromatography results for raw sugar samples

Sample name	Sample Type	Mill	Glucose %	Fructose %	Sucrose %
RS 0152	Raw sugar	Mill 7	0.86	0.96	90.62
RS 0040	Raw sugar	Mill 7	0.56	0.64	98.77
RS 0027	Raw sugar	Mill 7	0.13	0.15	89.23

Samples were analysed by ICP-AES for major and trace elements and Dumas combustion for carbon and nitrogen (Table 33). Sample *RS 0027* had much higher calcium, iron and manganese levels than the other two samples and *RS 0040* had much lower potassium, magnesium and sodium levels.

Table 33: ICP-AES results for raw sugar samples

Sample name	Sample Type	Mill	Ca mg/L	Fe mg/L	K mg/L	Mg mg/L	Mn mg/L	Na mg/L	C %	N %
RS 0152	Raw sugar	Mill 7	99.5	1.13	181.7	40.25	0.34	11.63	42	0.028
RS 0040	Raw sugar	Mill 7	91.5	1.13	92.48	23.16	0.24	6.36	42	0.027
RS 0027	Raw sugar	Mill 7	167.7	2.77	187.6	57.55	0.79	12.08	42	0.040

A PCA was conducted on the wet chemistry characterisation data (mill wet chemistry, NIR predicted wet chemistry and analytical wet chemistry) to observe the correlations that exist between the techniques.

As was done previously, all data was autoscaled prior to analysis to give a mean of zero and standard deviation of one. Three samples are not typically enough to complete a PCA. To generate a PCA to interpret the relationships between the variables each sample was duplicated to make six individual

samples. This gave a PCA with two principal components and the results should be used as a guide only, as the model will be overfit. A biplot produced during the analysis is provided in Figure 108. As the data was the same, the duplicate sugar samples overlapped each other perfectly. The three sugar samples are independent across the two PCs, however PC 1 shows the change in pol as the gross feature. The *pol, ppol* and *sucrose%* values were fairly well correlated, as were the various moisture measurements. The distribution on PC2 mostly represented the change in the salt profile and the reducing sugars/sucrose relationship.

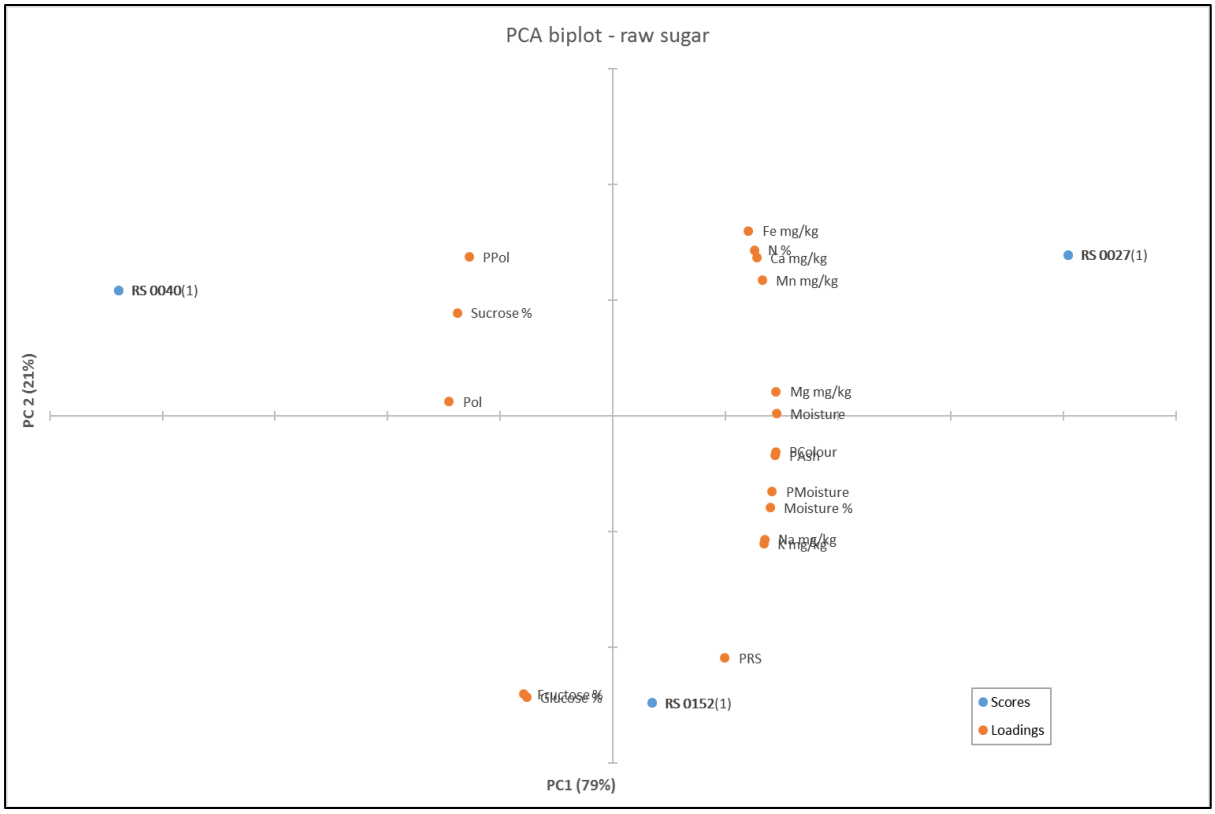

Figure 108: PCA biplot of all mill wet chemistry, NIR predicted wet chemistry and analytical wet chemistry for raw sugar samples

All three sugar samples were analysed by NIR and the spectra are provided in Figure 109. The three samples showed very consistent spectral patterns, with only slight variations in absorbance at the tips of each peak. Interestingly, the shift in absorbance does not appear to have the same pattern as the shift in major chemistry (pol/moisture). An annotated version of the spectra showing the NIR spectroscopic band assignments is provided in Figure 110. The major peaks in these spectra are attributed to water. Specifically, the peaks at 6300, 6969 and 7273 cm^{-1}. Other key peaks were related to $–CH_2$ or $–CH_3$-related stretching and bending.

The FT-IR spectra for the three raw sugar samples are provided in Figure 111. The minimal amount of moisture in the sample has resulted in extremely sharp, clean peaks. As with the NIR, the three sugar samples are closely aligned and only show slight variance in their absorbance at specific peaks. In the FT-IR spectra, however, the absorbance change trends in the same manner as the sucrose. A very comprehensive band assignment for the FT-IR spectra is provided in Figure 112.

This was adapted from a complete characterisation of pure sucrose (Brizuela et al., 2012) and was possible due to the very strong similarity between the spectra of pure sucrose and that of the raw sugar.

The TGA analysis (Figure 113) of raw sugar did not provide much additional information. The low moisture content meant no mass change up to 200 °C. At around 190 °C, the sugar likely melts (not observed as a mass loss). The sugar then begins caramelisation after 200 °C and undergoes similar complex reactions to those described for the molasses samples. The major mass losses are likely to be a result of water and carbon dioxide. The rate of the mass loss is much faster in the raw sugar and is likely due to the purity of the sample, relative to molasses.

The calibration coefficients for the G16.1 models for raw sugar are provided in Figure 114 to Figure 118. As with the molasses samples, the calibration coefficients are very active right across the spectrum and do not show any clear spectral features. The most prevalent region for all on the coefficient plots is between 7400 and 7143 cm^{-1} (1350 - 1400 nm), which includes a $–CH_3$ combination, a $–CH_2$ combination and a water combination (Figure 110). Due to the purity of the sample, this region is likely capable of explaining the major variance within the samples.
The correlation coefficients for reducing sugars, colour and ash have a similar gross structure, but small variations about 1220, 1475, 1575 nm set them apart.

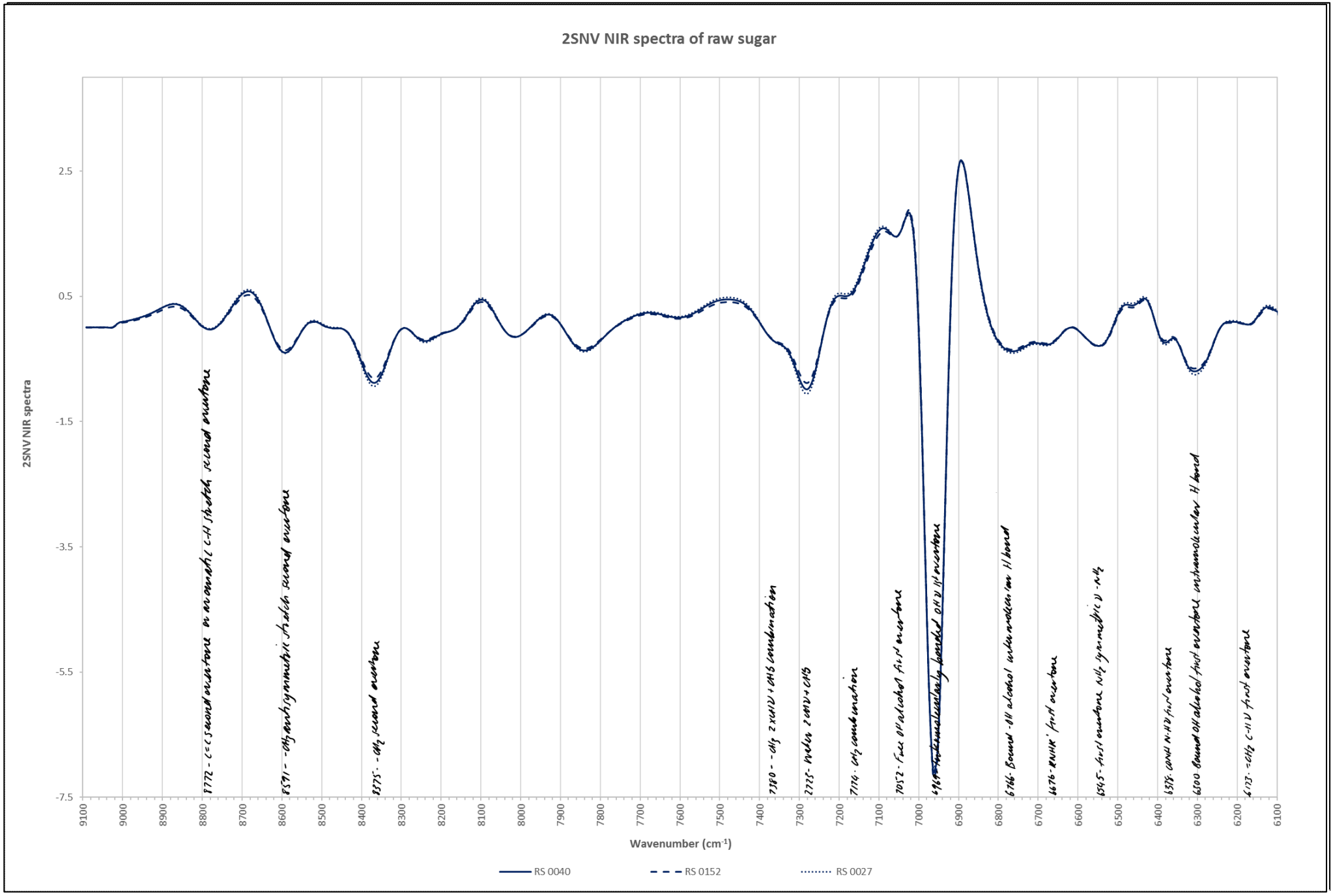

Figure 110: NIR spectra of raw sugar showing band assignments (2000)

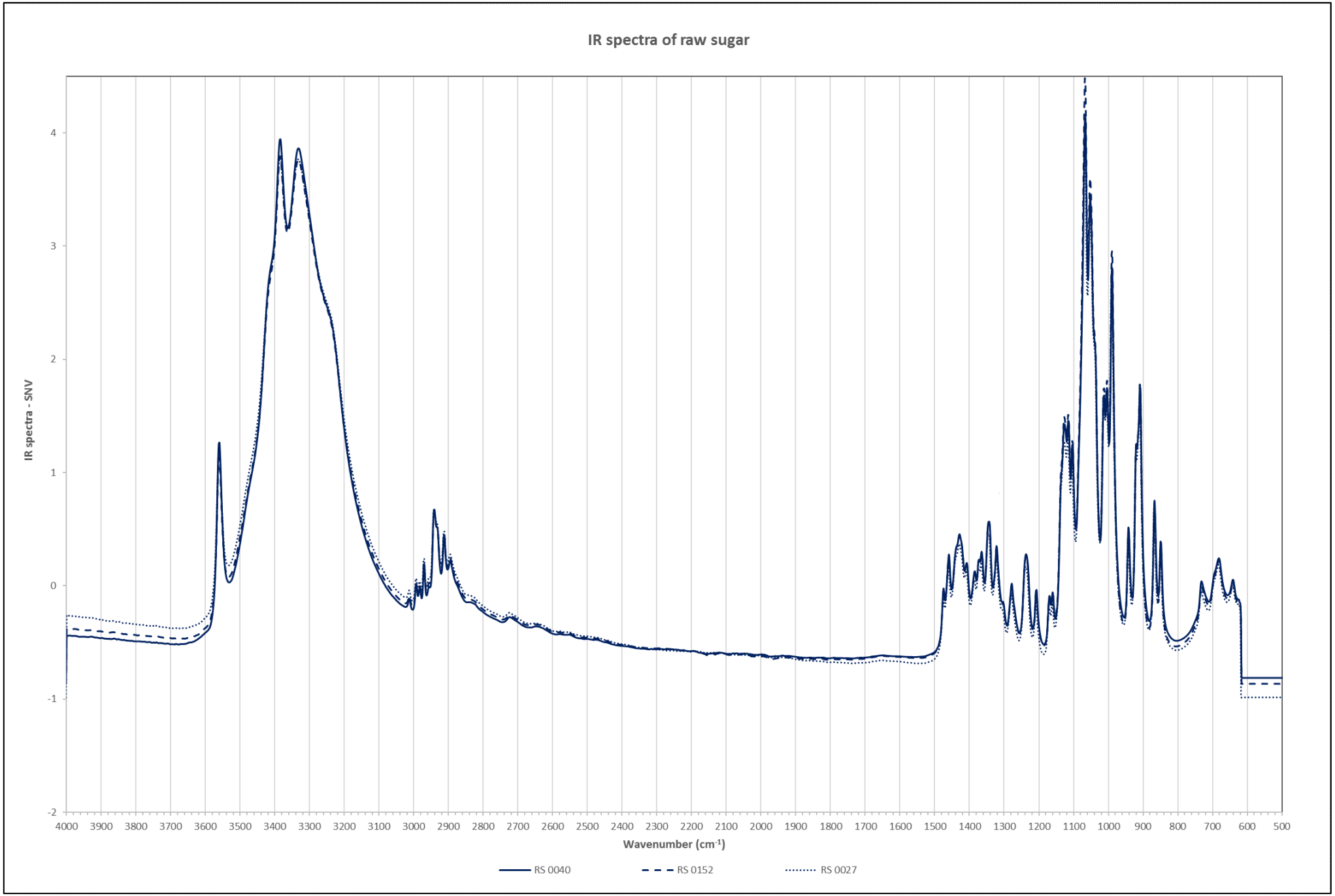

Figure 111: FT-IR spectra of raw sugar

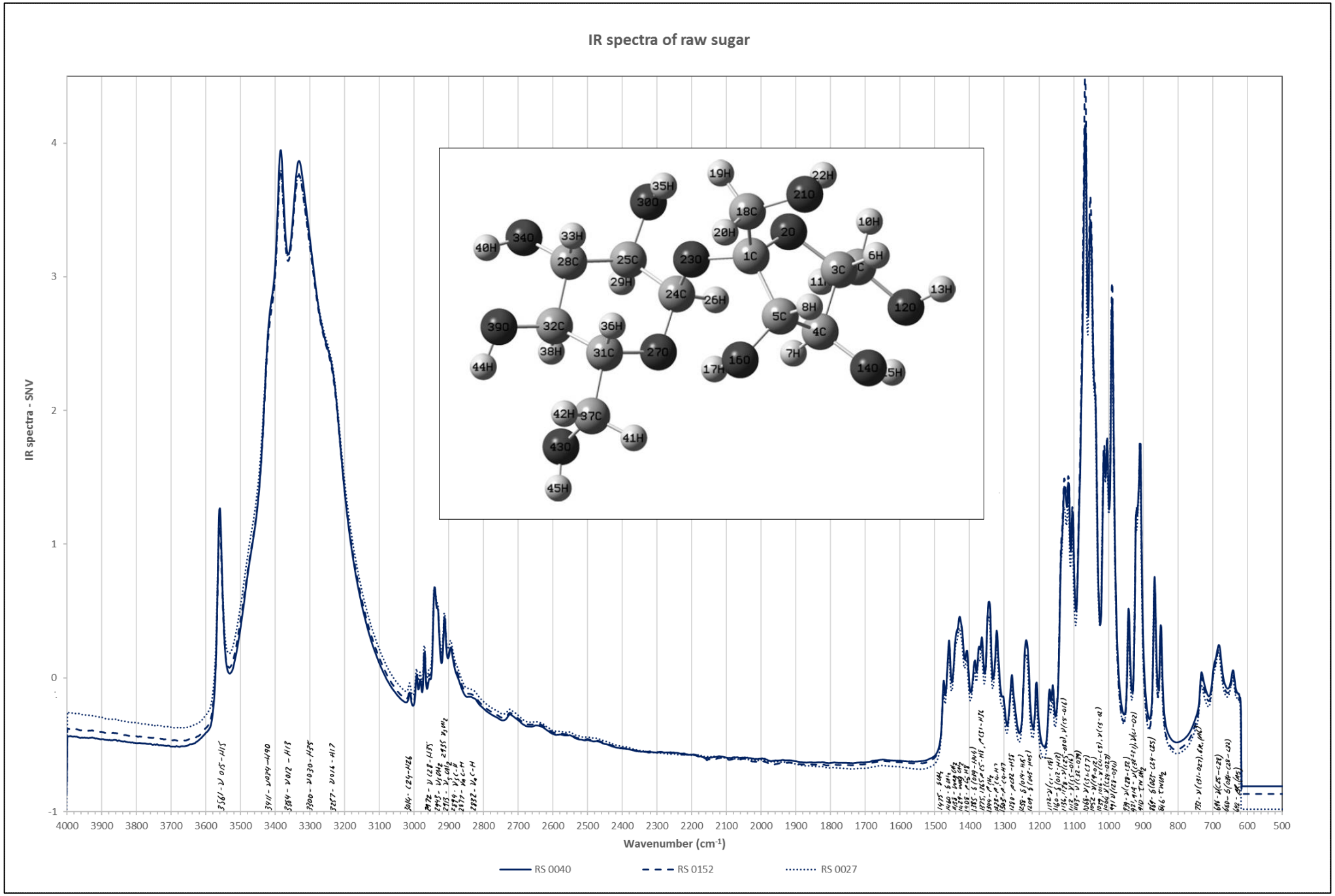

Figure 112: FT-IR spectra of raw sugar with band assignments showing inset the molecular structure of sucrose with labelled atoms (Brizuela et al., 2012, Williams and Fleming, 1995, Shenk et al., 2001, Nolasco and De Massaguer, 2006, Guilherme et al., 2015, Iwamoto et al., 1986, Mortari et al., 2012, Murray and Williams, 1987, Robert et al., 1993, 2000, Mlotkiewicz, 2005, Bilba and Ouensanga, 1996, Chaplin, 2000)

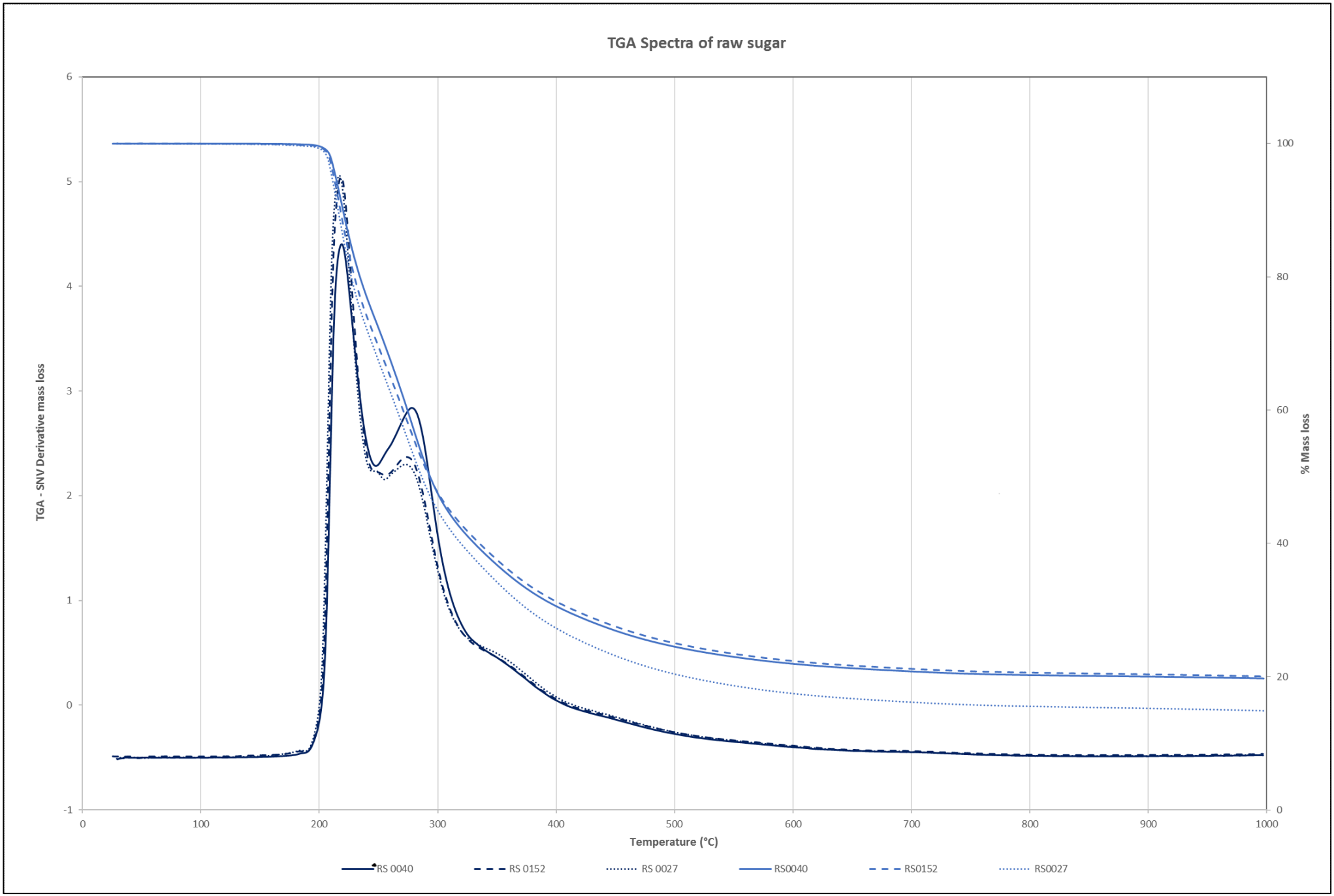

Figure 113: TGA spectra of raw sugar

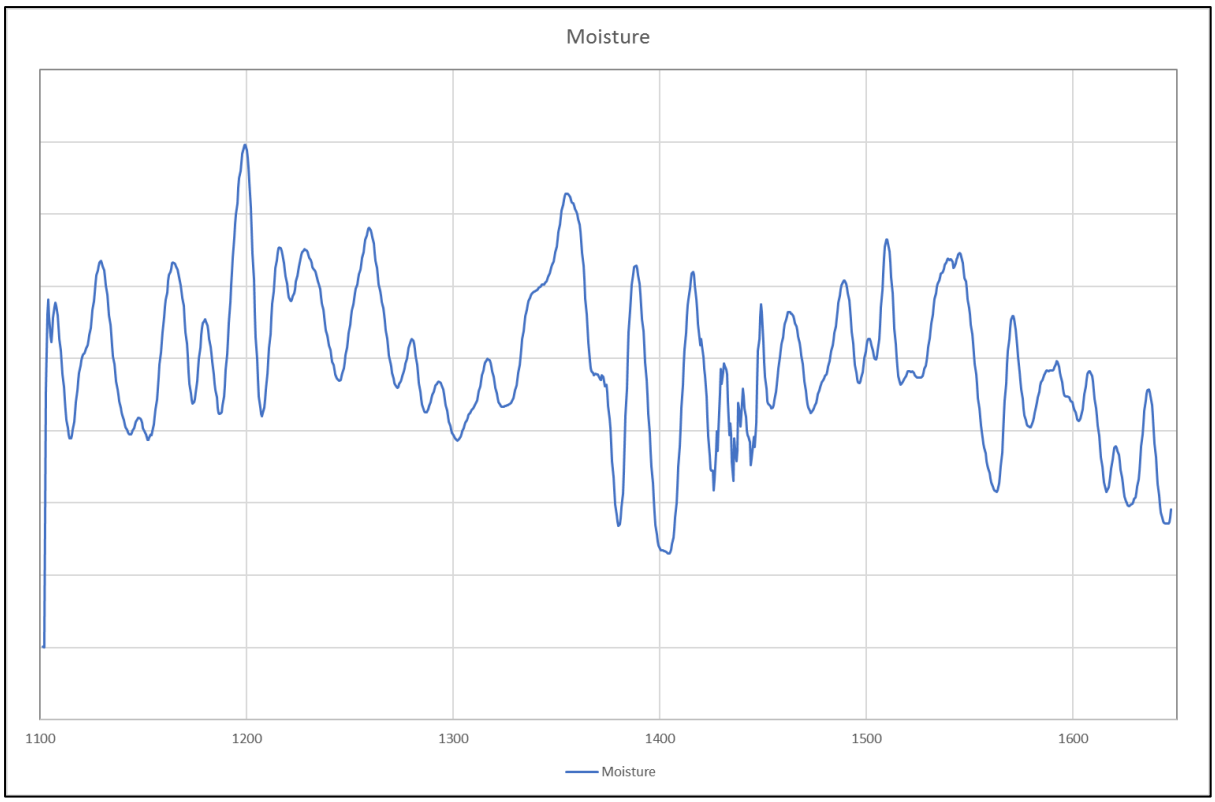

Figure 114: G16.1 calibration coefficient for raw sugar moisture

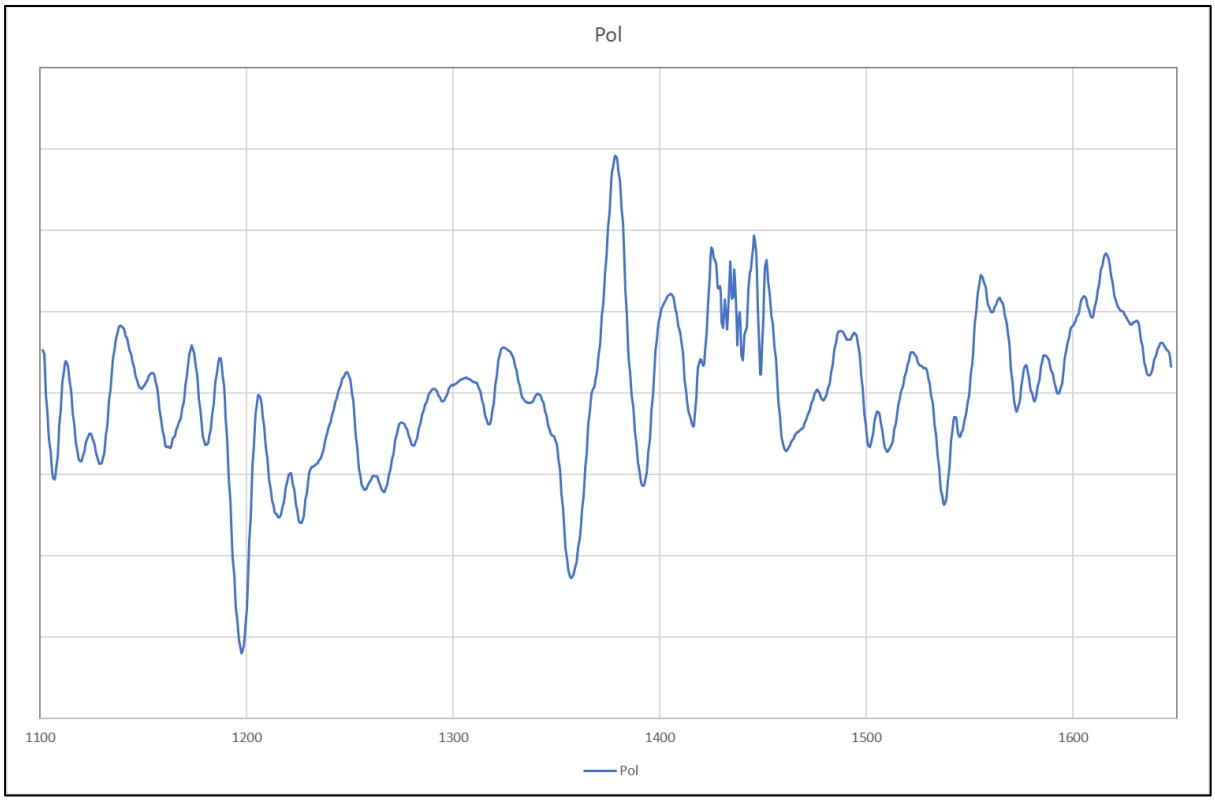

Figure 115: G16.1 calibration coefficient for raw sugar pol

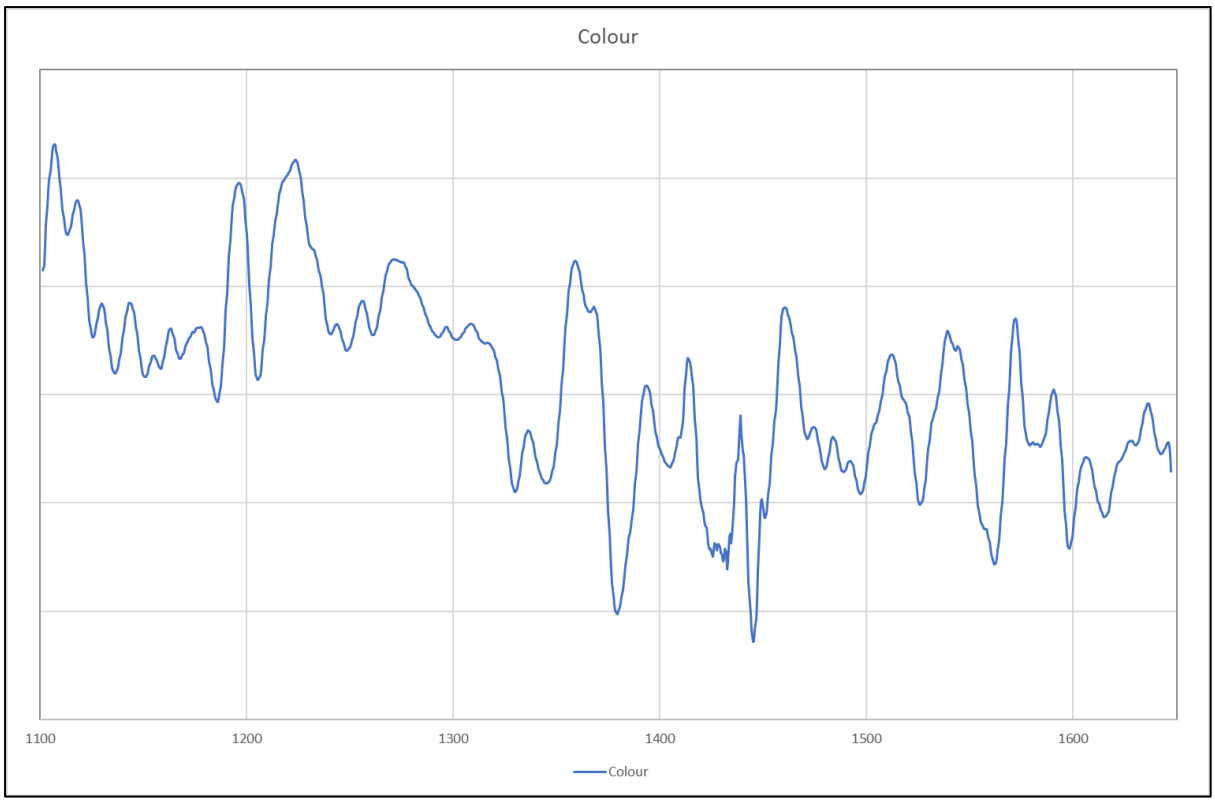

Figure 116: G16.1 calibration coefficient for raw sugar colour

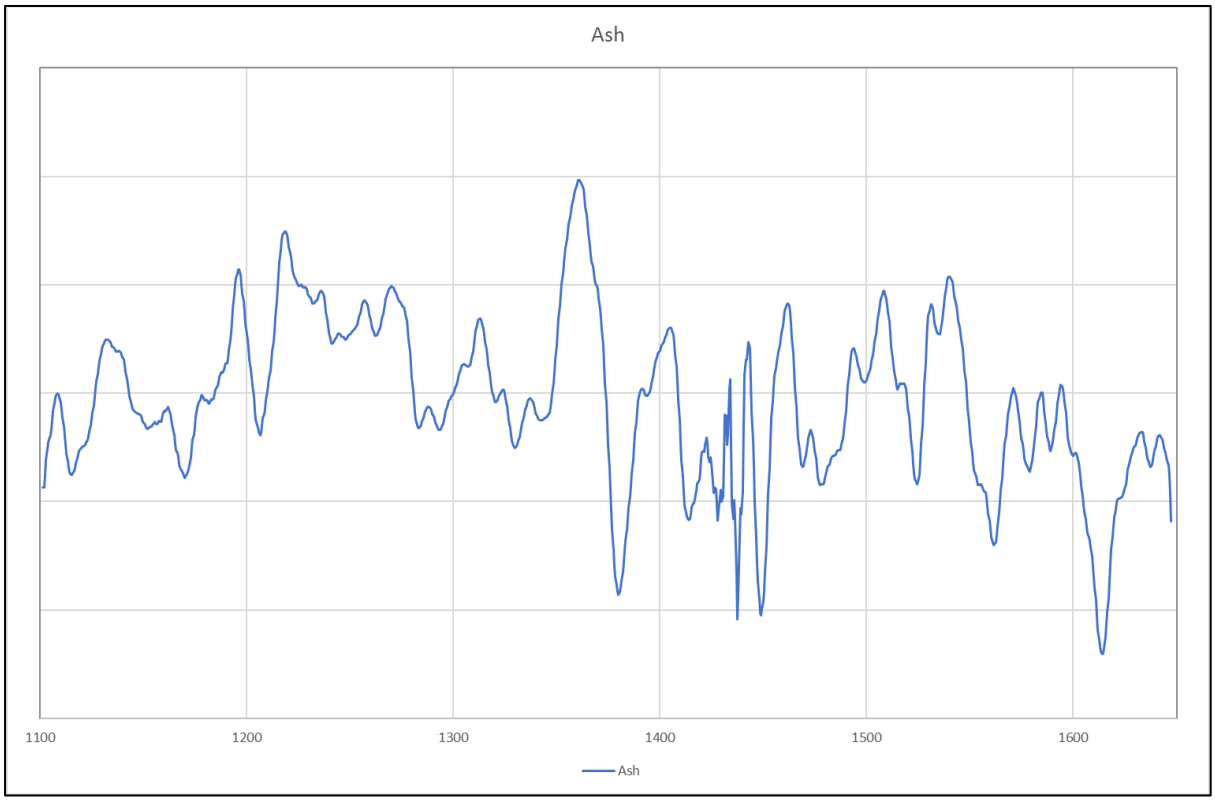

Figure 117: G16.1 calibration coefficient for raw sugar ash

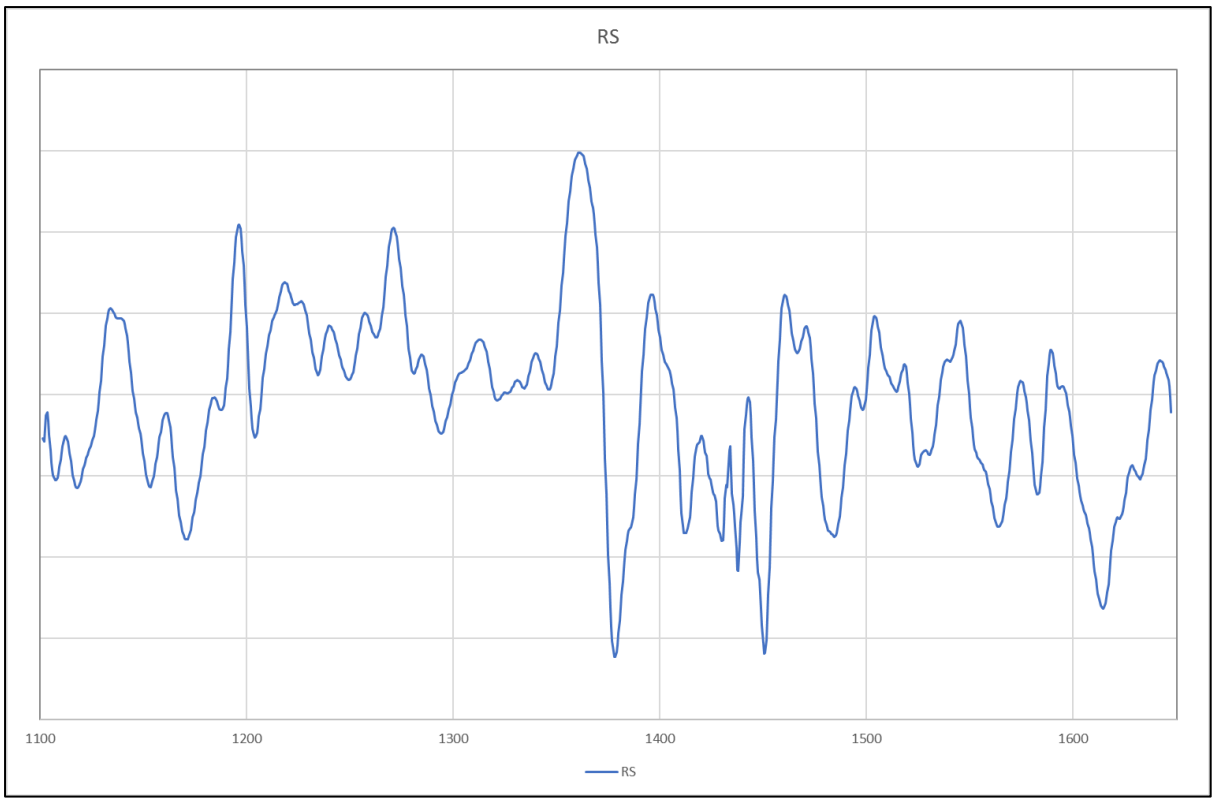

Figure 118: G16.1 calibration coefficient for raw sugar reducing sugars

6.2.1.3. Bagasse

Three bagasse samples were collected from Mill 11. Moisture and pol values were generated by the mill according to standard methods. Ash and extractives were measured at SRA's Indooroopilly laboratory (Table 34). Only Perten DA7250 spectra were available for these samples so NIR predicted wet chemistry results are not available. The bagasse samples varied in moisture and pol composition inversely. As expected the extractives correlate with the pol values.

Table 34: Mill and analytical wet chemistry results for bagasse

Sample name	Sample Type	Mill	Moisture %	Pol %	Ash %	Extractives %
Bag 0119	Bagasse	Mill 11	49.75	8.55	4.9	26.1
Bag 0125	Bagasse	Mill 11	46.8	1.06	4.6	6.1
Bag 0221	Bagasse	Mill 11	53.65	2.09	3.2	11.1

The NIR spectra of samples *Bag 0119* and *Bag 0221* are very similar, but *Bag 0125* shows variance in absorbance at most key wavelengths (Figure 119). This variance is likely due to a sharpening of the peaks due to a lower moisture content. The NIR spectra for bagasse show very active C-H stretching and bending vibrations that can be attributed to cellulose (8210, 7082, 6702 and 6440 cm^{-1}), lignin (8375, 8210, 7342, 7082, 6973, 6811 and 6640 cm^{-1}) and sucrose (6811 and 6640 cm^{-1}) (Keeffe, 2013, 2000, Murray and Williams, 1987, Iwamoto et al., 1986).

The FT-IR spectra of the three bagasse samples in Figure 120 show moderate differences in the absorbance at most frequencies, but no frequency shifting is observed. The variation in absorbance is likely due to the variation of moisture and sugars in the sample. This also explains the appearance of sharp peaks between 1200 and 1000 cm^{-1} for sample *Bag 0125*, which has a lower moisture content than the other samples.

The sharp peaks observed in this region can be associated with the C-O stretching of sucrose, glucose and fructose, as well as lignin and hemicellulose (Guilherme et al., 2015, Williams and Fleming, 1995, Bilba and Ouensanga, 1996).

The TGA spectra for bagasse are more complex than that seen for molasses and raw sugar and the major mass losses occur at higher temperatures (Figure 121). Samples *Bag 0221* and *Bag 0125* have a very similar response to the pyrolysis, whereas sample *Bag 0119* has a much greater mass reduction at 200 °C and undergoes subsequent mass losses at slightly lower temperatures than the other two samples. This is most likely a result of the much higher sugar loading of sample *Bag 0119*. As observed in the TGA for raw sugar (Figure 113) and molasses (Figure 104), mass loss associated with sugar is observed at around 200 °C. This region shows a reduction in mass that trends with the pol and extractives values of each sample. The subsequent mass losses at approximately 290 °C and 345 °C comprise a significant portion of the sample degradation during pyrolysis. The mass loss at 290 °C is caused by the decomposition of hemicellulose (Mortari et al., 2012). The mass loss at 345 °C is associated with the decomposition of cellulose (Mortari et al., 2012, Munir et al., 2009). Following this is the slow decomposition of lignin, which is difficult due to the complexity of the product (Mortari et al., 2012). The variation in mass loss at 290, 345 and 410 °C is independent of extraction efficiency. Instead, sample *Bag 0221* shows a much lower lignin content than the other two samples. These samples were not subjected to the full biomass compositional analysis by wet chemistry, nor are such calibrations currently available on the DA1650. This information may provide insight into the millability of canes as they come into the factory, allowing better adjustment of the milling train to improve extraction. Further investigation in the future may be worthwhile.

The G16.1 coefficient plots for bagasse are provided in Figure 122 to Figure 126. Two separate models are used for pol in bagasse to minimise the error of the low pol final mill predictions. The correlation coefficients for primary (Figure 122) and final (Figure 123) mill pol are very similar, as would be expected. Key sucrose vibrations are present at 6811 cm^{-1} (1468 nm) and 6640 cm^{-1} (1506 nm). Additionally major vibrations are present at 8375 cm^{-1} (1197 nm), 8210 cm^{-1} (1217 nm) and 6702 cm^{-1} (1491 nm) that relate to cellulose and lignin vibrations. The brix calibration coefficient in Figure 124 is relatively featureless, but shows activity in the water region at 7418 cm^{-1} (1348 nm) and the C-H combination and O-H stretching region between 7143 cm^{-1}(1400 nm) and 6900 cm^{-1} (1450 nm). The fibre (Figure 125) and moisture (Figure 126) both have strong activity between 7143 cm^{-1}(1400 nm) and 6900 cm^{-1} (1450 nm) as well as the N-H stretching region between 6452 cm^{-1} (1550 nm) and 6079 cm^{-1} (1650 nm). The moisture coefficient also has active peaks in the higher frequency end of the spectrum, as would be expected.

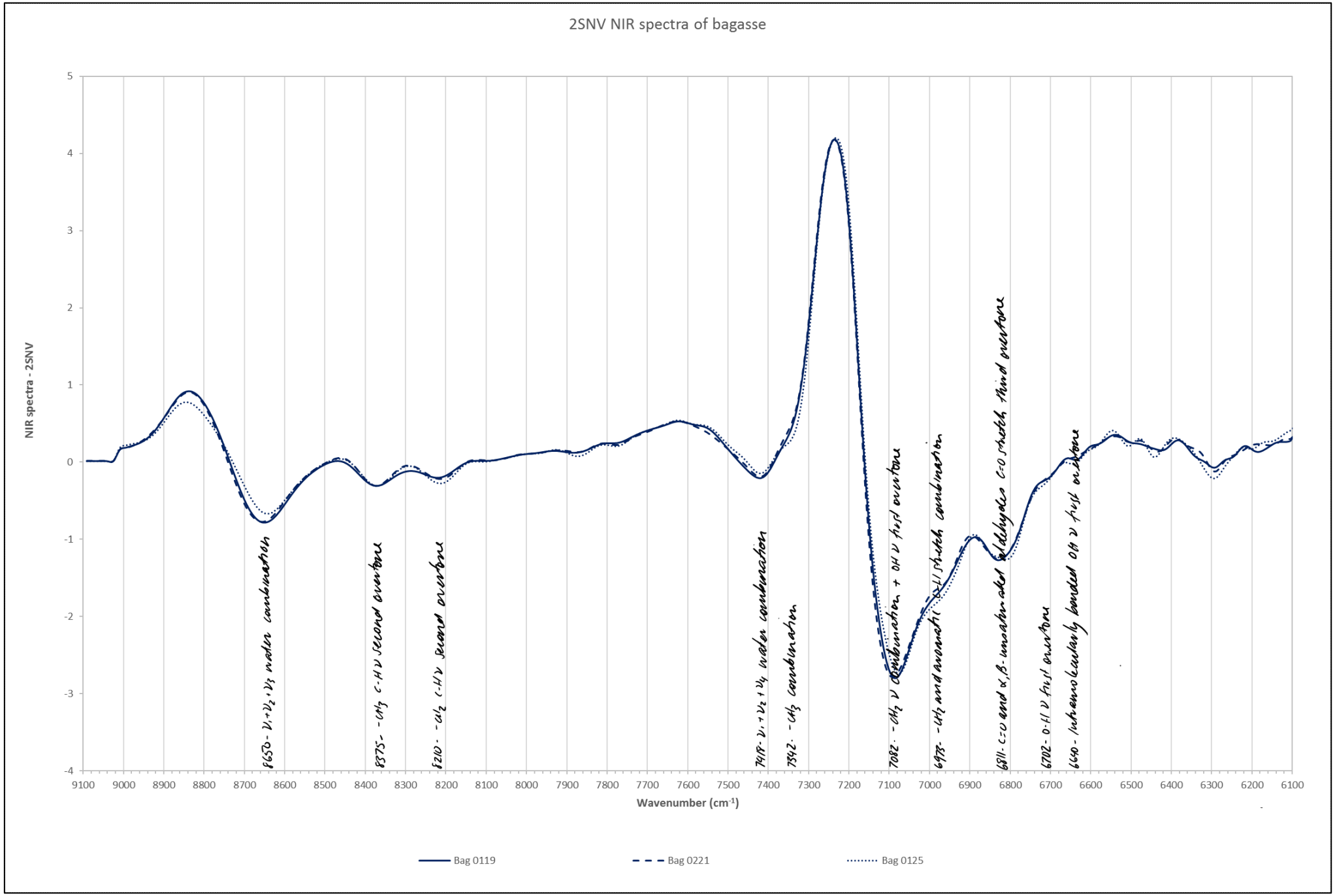

Figure 119: NIR spectra of bagasse samples showing mark-up (Keeffe, 2013, 2000)

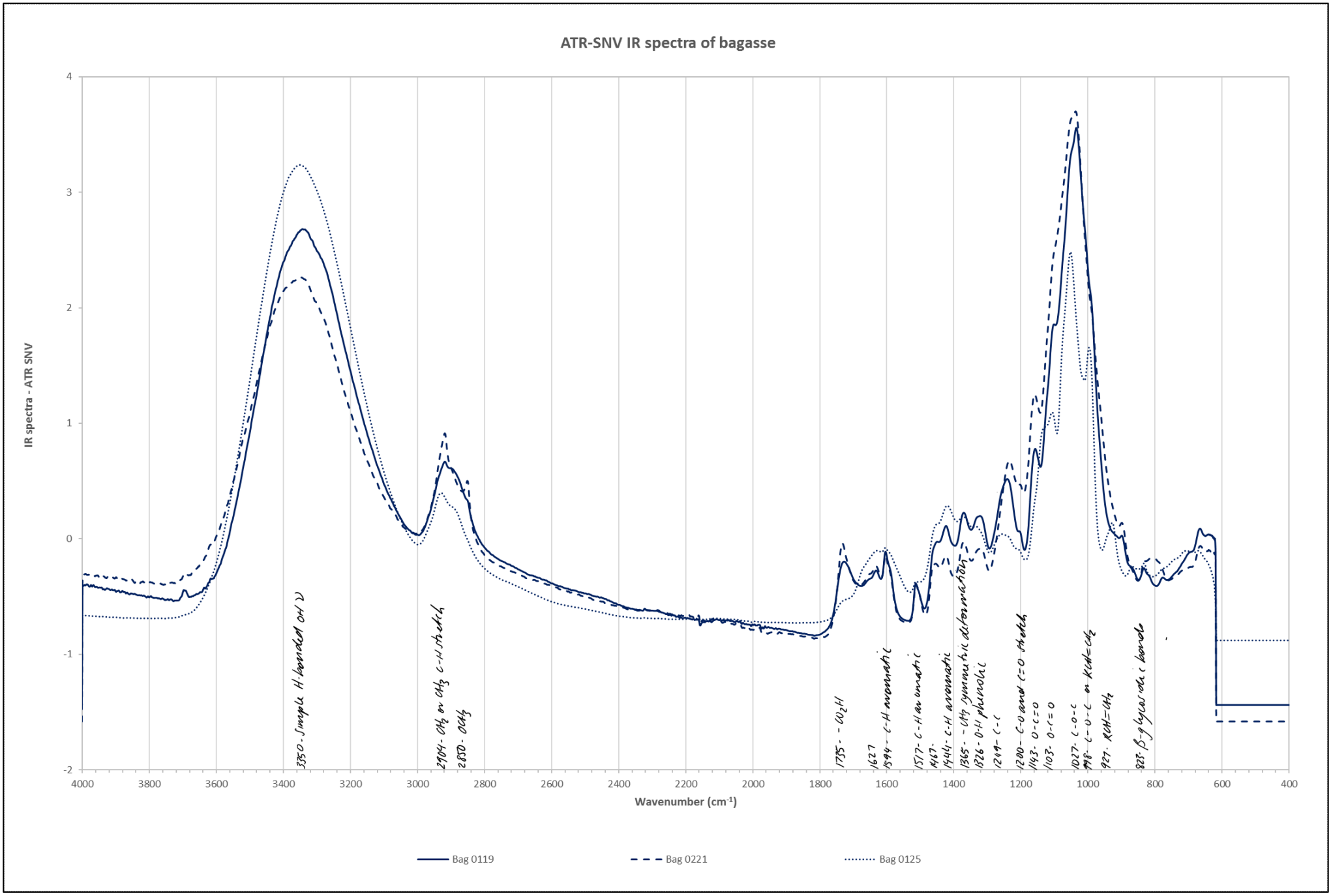

Figure 120: FT-IR spectrum of bagasse showing mark-up (Williams and Fleming, 1995)

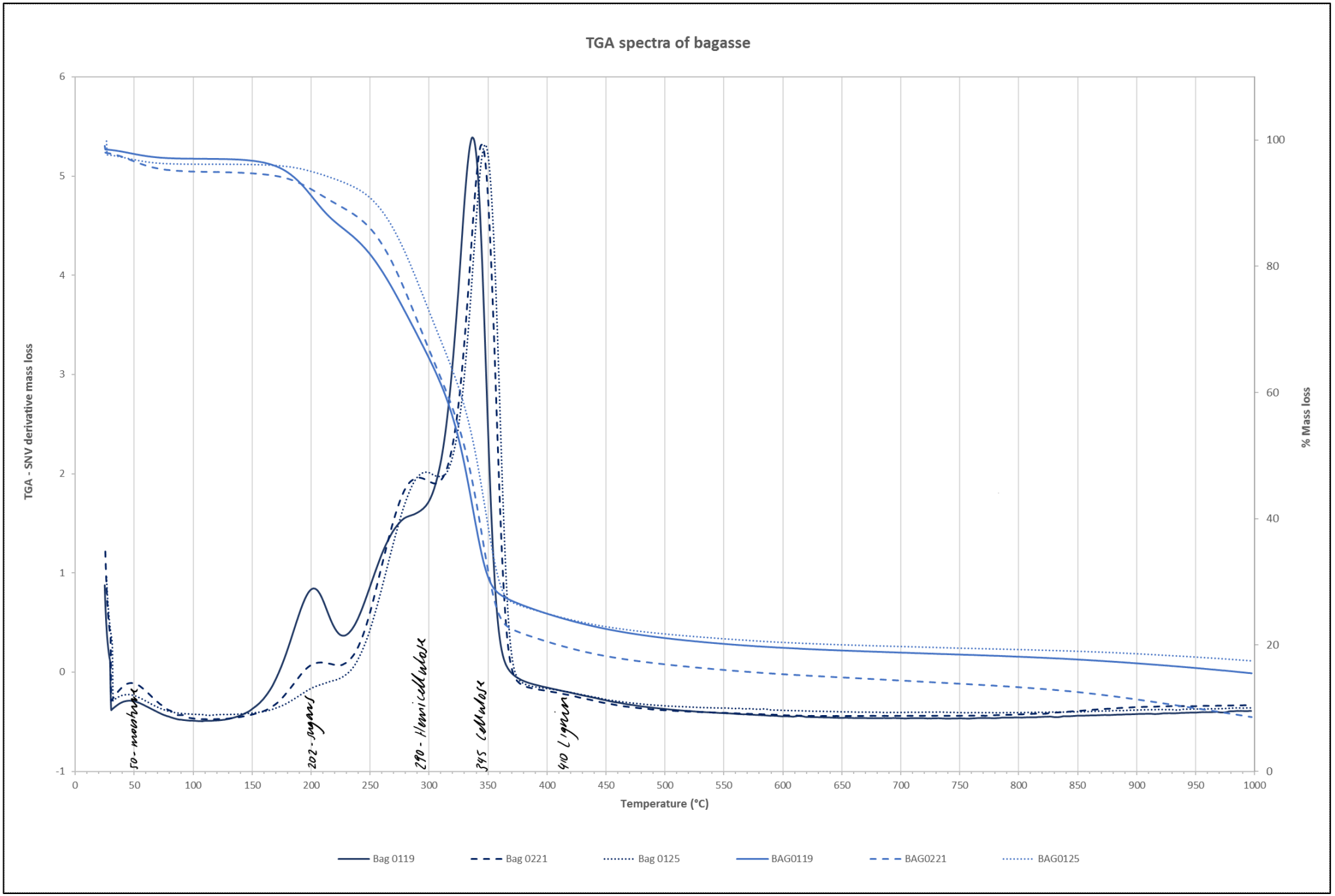

Figure 121: TGA spectrum of bagasse showing mark-up (Munir et al., 2009, Murray and Williams, 1987)

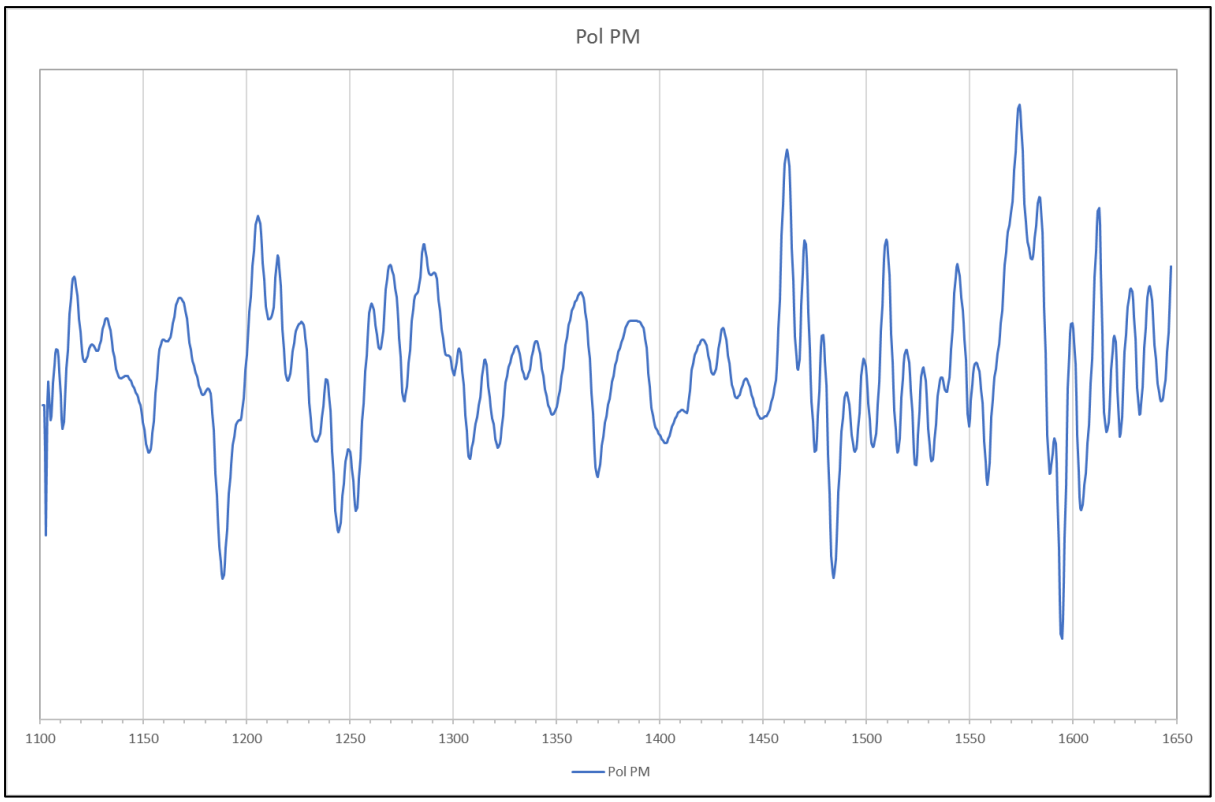

Figure 122: G16.1 calibration coefficient for bagasse pol primary mill

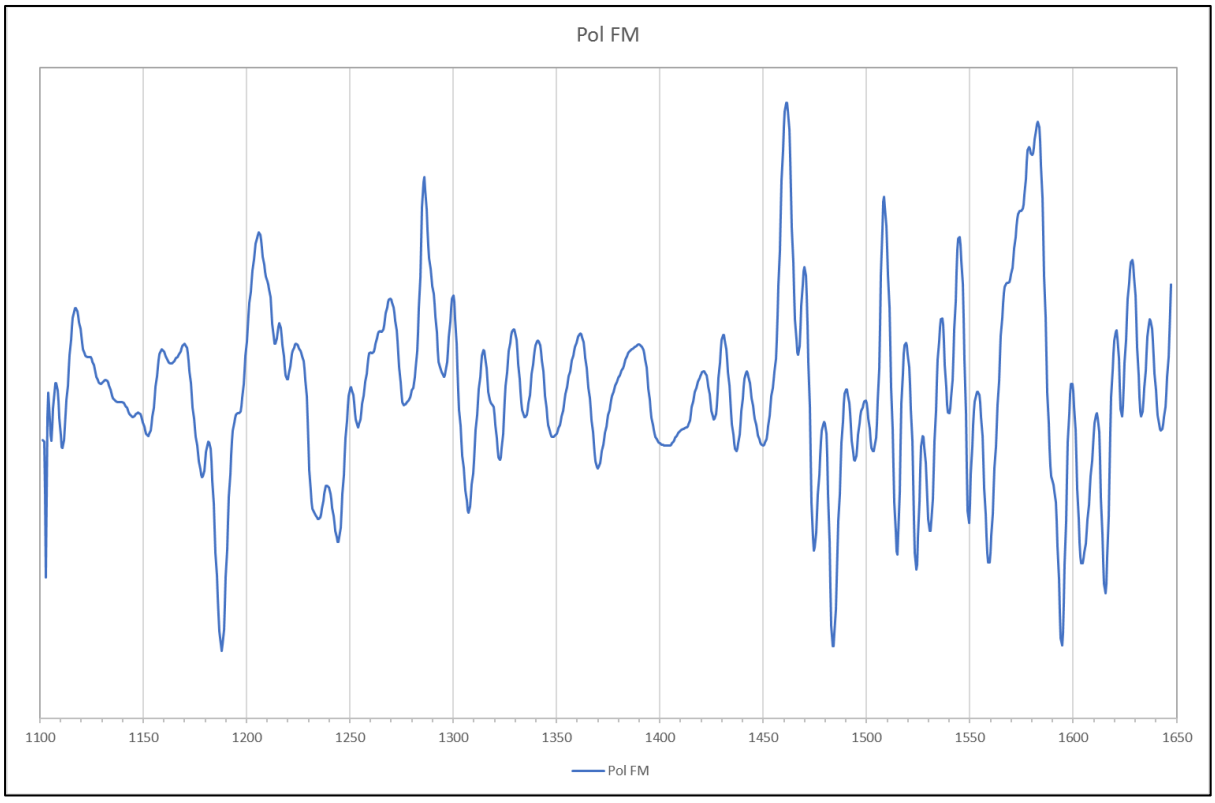

Figure 123: G16.1 calibration coefficient for bagasse pol final mill

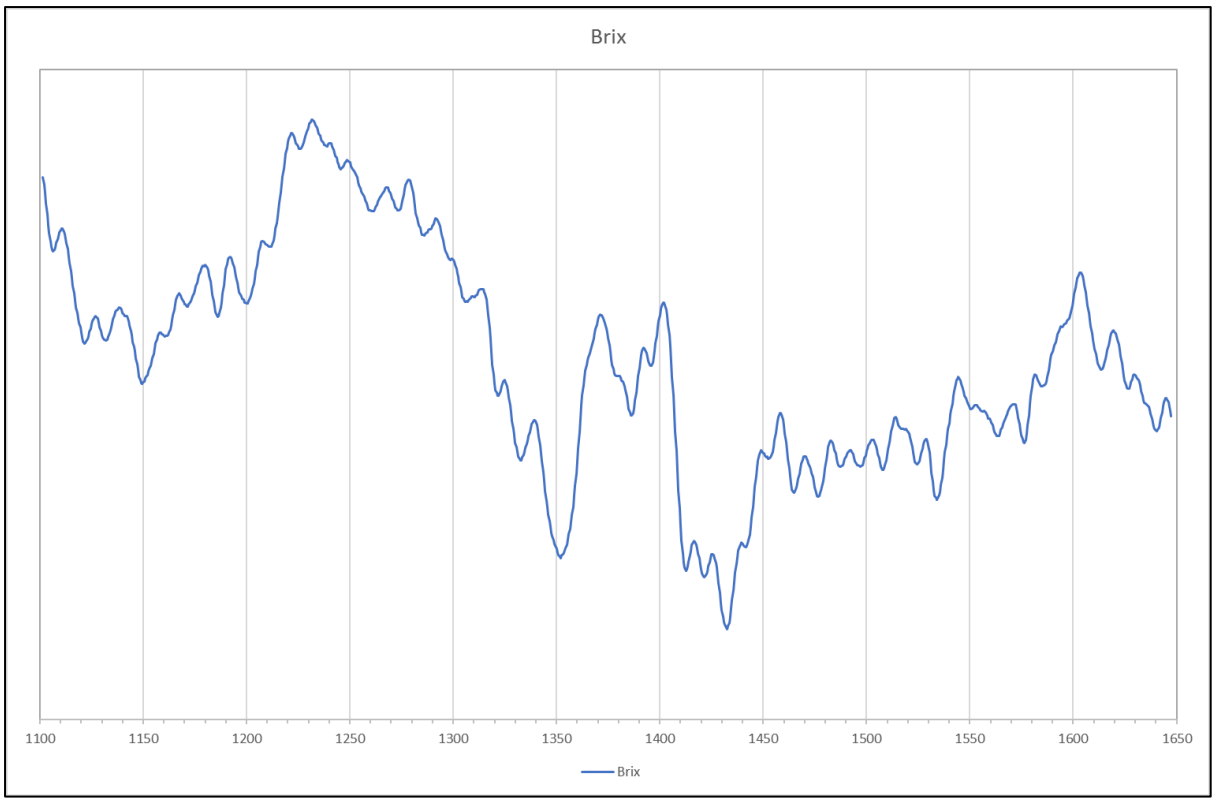

Figure 124: G16.1 calibration coefficient for bagasse brix

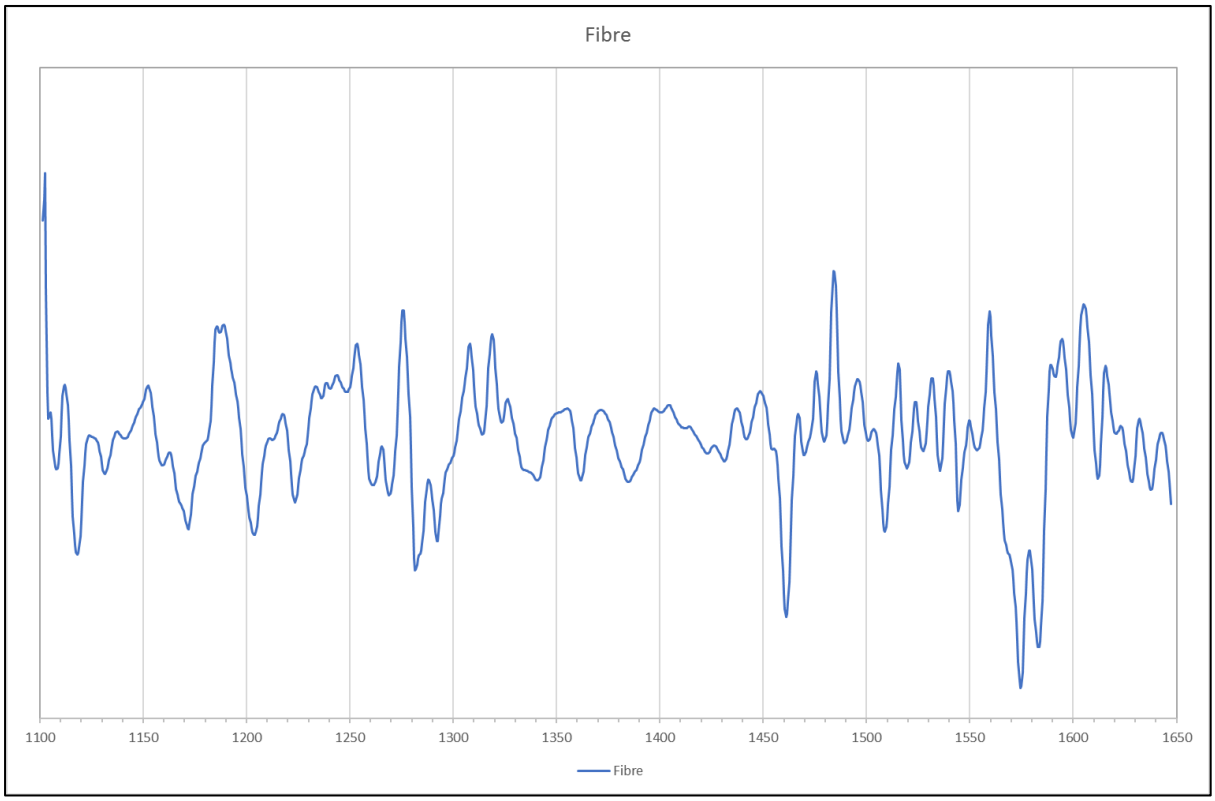

Figure 125: G16.1 calibration coefficient for bagasse fibre

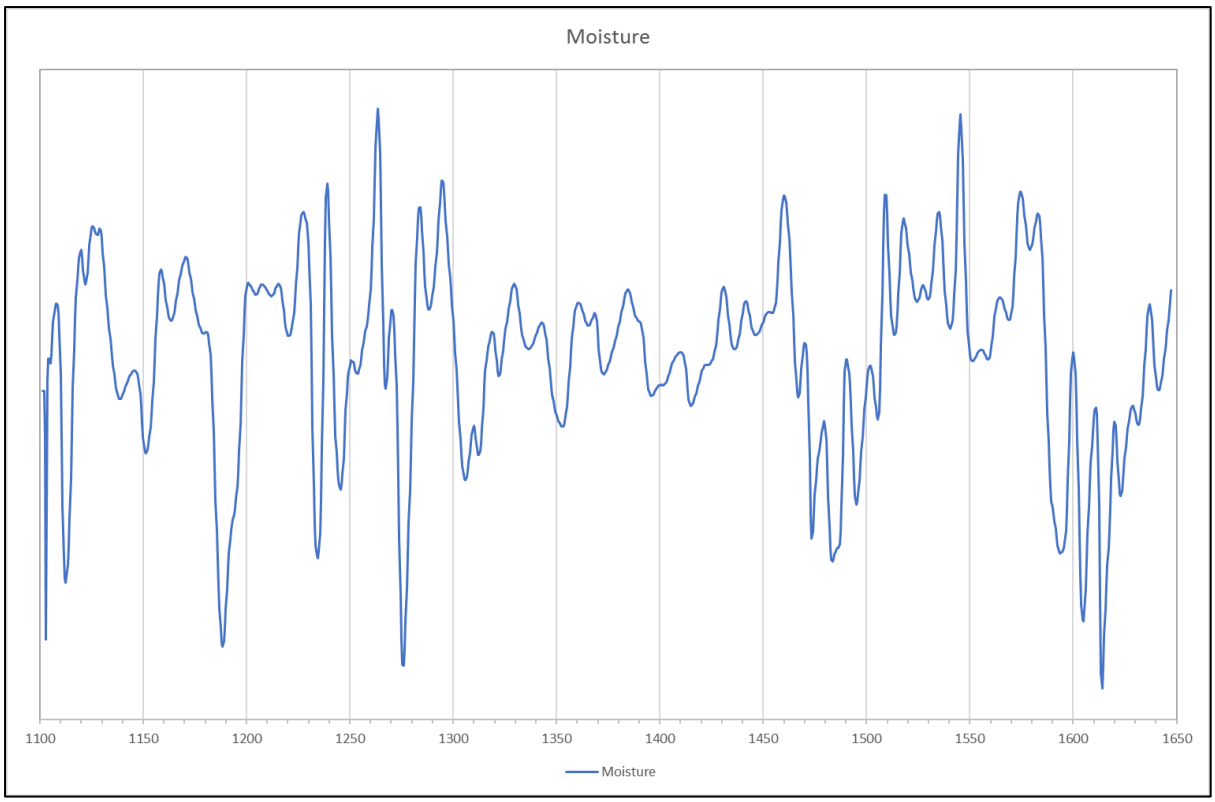

Figure 126: G16.1 calibration coefficient for bagasse moisture

6.2.1.4. Other factory products

Prepared cane, massecuite, magma, juice, syrup and mud were not characterised by additional spectroscopic techniques, but were subjected to some of the analytical wet chemistry methods. The results for these analyses are provided in Table 35.

Table 35: Characterisation data of other mill products

Sample number	Type	Product	Brix %	Pol degZ	Suc %	DS %	RS %	Ash %	CCS	Water %	%CC	IW	Fibre %	Moist %	Ash %	Glu %	Fru %	Suc %	Ca mg/L	Fe mg/L	K mg/L	Mg mg/L	Mn mg/L	Na mg/L	P mg/L	S mg/L	C %	N %	Extrvs %dm
Mas0015	A	Massecuite	93.9	80.0										6.5	2.90	1.43	1.21	84.7	10477	89.6	38314	3263	70.88	108	432	2242	38.7	0.17	
Mas0011	A	Massecuite	93.8	78.7										5.9	2.82	1.91	1.60	79.9	8960	84.7	30172	3174	50.59	266	502	2646	38.9	0.16	
Mas0236	A	Massecuite			75.6	87.9				12.1	42.9			9.3	3.41	2.00	1.66	74.9	11526	83.4	27382	7422	31.00	2327	620	6719	36.4	0.23	
Mas0003	A	Massecuite	94.8	77.7										5.9	2.86	2.36	1.95	77.3	9580	97.2	28158	3322	67.92	181	479	2449	38.2	0.19	
Mas0089	A	Massecuite			81.2	91.8				8.2	54.6			7.9	3.14	2.12	1.58	78.4	10293	82.2	29551	8080	61.20	3849	725	8077	38.3	0.17	
Mas0143	A	Massecuite			91.1	93.2								7.4	2.99	1.11	1.01	80.4	9947	214.2	36831	3105	116.1	1227	1108	3407	38.2	0.22	
Mas0249	A	Massecuite			79.4	90.6				9.4	47.6			6.3	3.32	1.79	1.36	79.7	14066	78.5	32706	9245	47.54	2074	731	8459	38.1	0.22	
Mas0010	B	Massecuite	94.9	71.4										6.2	4.88	3.44	3.09	72.9	13485	110.4	47446	5083	88.33	393	738	4345	37.9	0.27	
Mas0038	B	Massecuite	92.7	68.6										8.0	3.72	3.85	3.45	71.5	8470	91.0	25329	3050	51.47	133	399	2735	37.6	0.24	
Mas0017	B	Massecuite	95.1	70.3										6.9	4.61	3.38	2.95	68.6	13590	121.5	43338	5833	103.7	350	675	4433	37.4	0.32	
Mas0095	B	Massecuite			72.7	90.4				9.6	36.7			8.1	4.56	2.51	2.13	73.6	2921	19.5	8935	2556	9.69	560		2410	37.3	0.28	
Mas0243	B	Massecuite			75.2	91.9				8.1	47			5.6	4.77	2.85	2.41	75.3	13726	77.9	39465	9656	44.04	2681	775	10021	37.9	0.27	
Mas0060	B	Massecuite			77.6	92.3				7.7	51			5.0	4.70	2.01	1.77	77.2	9883	56.0	28274	7202	27.74	2147	655	6989	38.0	0.26	
Mas0009	C	Massecuite	99.9	59.8										1.4	8.62	5.07	5.54	45.2	12349	116.4	47313	4835	81.95	289	607	4307	38.0	0.47	
Mas0007	C	Massecuite	100.5	58.2										3.2	7.90	5.45	6.18	58.7	10285	115.4	36196	4066	73.82	290	509	3668	37.9	0.47	
Mas0005	C	Massecuite			78.1	91.6								0.9	8.09	6.02	6.06	60.2	10305	99.6	35862	4628	81.72	243	490	3729	38.2	0.48	
Mas0090	C	Massecuite			58.1	90.1				9.9	33.7	3.2			9.85	3.86	3.80	59.2									37.0	0.58	
Mas0088	C	Massecuite			61.7	91.4				8.6	38.7	3.4		3.9	9.00	4.27	4.34	61.1	9114	60.1	27985	7190	32.20	1599	559	7841	36.8	0.48	
Mas0233	C	Massecuite			59.2	91.8				8.2	35.2	3.9		3.9	9.57	0.11	0.11	60.8	12541	80.1	38552	8814	37.29	2275	702	9869	36.6	0.51	
Mas0161	C	Massecuite			63	94.6								1.0	7.89	4.84	5.49	61.6	5919	55.6	22530	2071	47.98	225	427	2460	38.4	0.41	
Mas260	C	Massecuite			57.6	91								4.1	9.48				6959	37.3	17771	5454	19.28	945	351	4168	36.6	0.58	
Mag 0043		Magma	87.0	79.0						9	33.7	3.7		13.6	2.25	0.59	0.32	78.2	1654	10.9	4829	981	8.80	144		1065	35.1	0.15	
Mag 0113		Magma	80.2	85.7										14.5	1.40	0.38	0.41	80.5	960	5.5	3101	643	4.29	63		707	35.1	0.12	
Mag 0108		Magma	78.8	88.2										11.8	2.58	0.70	0.76	78.8	1993	8.5	5681	1264	9.01	131		1312	35.3	0.18	
Mag 0010		Magma	99.6	78.7										7.5	2.62	1.93	2.23	78.0	1932	20.9	6079	906	11.98	99		679	37.8	0.18	
Mag 0083		Magma			82.3	89.8								8.4	2.09	1.21	1.31	83.6	1471	27.9	3958	790	9.24	659		1181	37.9	0.12	
Mag 0085		Magma			84.2	91.3								6.8	1.88	1.04	1.12	85.8	1404	16.2	4142	685	6.07	197		974	38.1	0.12	
Mag 0075		Magma			86.7	92.3								6.5	1.18	0.94	0.04	86.8	882	8.2	3027	484	2.75	89		699	38.6	0.10	
Mag 0100		Magma			77.9	83.9				16.1				12.6	1.75	0.76	0.82	79.7	1333	8.3	2969	930	4.07	238		767	34.9	0.12	
Syr 0062		Syrup	68.1	61.8										31.7	1.96	0.75	0.69	58.0	1263	5.1	3934	874	14.30	123		948	27.6	0.11	
Syr 0055		Syrup	67.5	60.3										30.5	2.12	0.81	0.72	56.9	1292	4.2	4566	841	6.41	65		1005	27.4	0.12	
Syr 0124		Syrup	33.4	30.2										31.5	2.07	0.82	0.72	57.9	1377	4.1	3869	854	10.03	118		899	26.7	0.14	
Syr 0072		Syrup	73.5	63.8	64	71.1	3.11	1.95						24.6	1.98	1.59	1.31	59.9	1415	13.2	4054	442	11.02	56		516	29.4	0.12	
PC 0009		Prep. cane						5.36					17.97	8.3	10.80														50.1
PC 0035		Prep. cane						0.87					11.94	7.8	3.20														63.4
PC 0046		Prep. cane						1.56					14.03	6.8	4.60														62.1
JF 0001	FEJ	Juice	20.6	17.2				1.51	12.17										163		1466	336		154	183	263	8.9	0.06	
JF 0007	FEJ	Juice	17.5	14.4				2.93	9.77										70		556	210		56	91	89	6.6	0.06	
JF 0127	FEJ	Juice	22.5	80.2				1.06	13.22										106		1147	292		247	205	197	9.3	0.04	
JE 0032	ESJ	Juice	14.9	15.1															287		1035	185		31	19	230	6.2	0.04	
JE 0034	ESJ	Juice	24.9	12.7															317		966	178		39	21	210	6.1	0.03	
JE 0035	ESJ	Juice	14.0	12.3															238		885	162		32	16	210	5.6	0.02	
JF 0046	FEJ	Juice	24.7	22.1					16.26										228		1315	387		5	92	338	10.7	0.07	
J4 0077	M4	Juice	1.6	1.2															13		146	18		6	19	27	0.7	0.01	
JE0096	ESJ	Juice	12.8	11.0															148		904	118		24	12	174	5.0	0.03	
MM 0015		Mill mud	2.9	1.1										76.3															
MM 0112		Mill mud	3.6	1.6										77.4															
MM 0109		Mill mud	7.1	1.9										78.4															

Suc: sucrose, DS: dry substance, RS: reducing sugars, CCS: commercial cane sugar, %CC: % crystal content, IW: insoluble to water ratio, Fru: fructose, Glu: glucose, Ca: calcium, Fe: iron, K: potassium, Mg: magnesium, Mn: manganese, Na: sodium, P: phosphorus, S: Sulphur, C: carbon, N: nitrogen, Extrac.: extractives

6.2.2. Development of molecularly-targeted calibration models

Based on the information obtained in Section 6.2.1.1, calibrations with reduced wavelength ranges were developed using the wavelength regions described in Table 28. The aim of this was to see whether simplifying the model (minimising the number of variables) to capture only specific wavelengths known to be related to the chemistry of interest, would improve the accuracy and robustness of the models.

Models were developed for sucrose, dry substance, brix, pol, ash and dry substance and validated during a trial at Mill 2 and are called Reduced Global 16.1 (R G16.1). Mill 2 only complete true purity analyses for their molasses samples and consequently, validation could only occur for sucrose and dry substance models. The performance statistics for the models are provided in Table 36 and calibration and validation plots (where available) are provided in Figure 127. For comparative purposes, the performance statistics for the latest molasses calibration suite are provided in Table 37 and calibration and validation plots (where available) are provided in Figure 128. The same samples were used for validation of the two different calibration sets and therefore, provide a direct comparison of model performance.

Table 36: Calibration and validation statistics for molasses Reduced Global 16.1 model

Constituent name	Calibration statistics									Validation statistics			
	SEC	R^2	Mean	SD	Min	Max	N	ECL	F	N	R^2	Bias	SEP
Brix % Molasses	1.96	0.94	82.32	8.13	12.40	99.40	1907	2.35	10				
Pol % Molasses	5.10	0.73	46.03	9.89	26.90	81.14	1516	6.12	3				
Dry Substance % Molasses	0.51	0.91	76.40	1.68	68.28	92.01	2145	0.61	9	101	0.10	0.47	2.78
Sucrose % Molasses	4.79	0.74	42.38	9.33	30.26	92.34	2700	5.75	16	98	0.16	-7.72	15.11
Ash % Molasses	0.46	0.56	13.43	0.69	11.58	15.34	386	0.55	10				
Reducing Sugars % Molasses	0.97	0.86	12.24	2.54	8.16	17.59	502	1.16	6				

SEC – Standard error of calibration, SD – standard deviation, N – number of samples in the dataset, ECL – Error control limit, F – number of factors in the model, SEP – Standard error of prediction

Table 37: Calibration and validation statistics for molasses Global 16.1 model

Constituent name	Calibration statistics									Validation statistics			
	SEC	R^2	Mean	SD	Min	Max	N	ECL	F	N	R^2	Bias	SEP
Brix % Molasses	0.94	0.98	82.51	7.55	12.40	99.40	1799	1.13	12				
Pol % Molasses	1.44	0.98	44.53	10.65	26.37	81.14	1577	1.72	14				
Dry Substance % Molasses	0.26	0.97	76.43	1.62	68.28	92.01	1954	0.31	13	101	0.69	0.64	0.75
Sucrose % Molasses	1.22	0.97	41.92	8.97	31.43	92.34	2579	1.47	16	98	0.93	-0.69	1.23
Ash % Molasses	0.30	0.82	13.40	0.71	11.18	15.34	364	0.36	8				
Reducing Sugars % Molasses	0.86	0.88	12.21	2.50	8.16	17.31	513	1.03	8				

SEC – Standard error of calibration, SD – standard deviation, N – number of samples in the dataset, ECL – Error control limit, F – number of factors in the model, SEP – Standard error of prediction. Bias and SEP have been calculated on outlier-free data.

Figure 127: Calibration and validation (where available) plots of the R G16.1 calibration suite for molasses

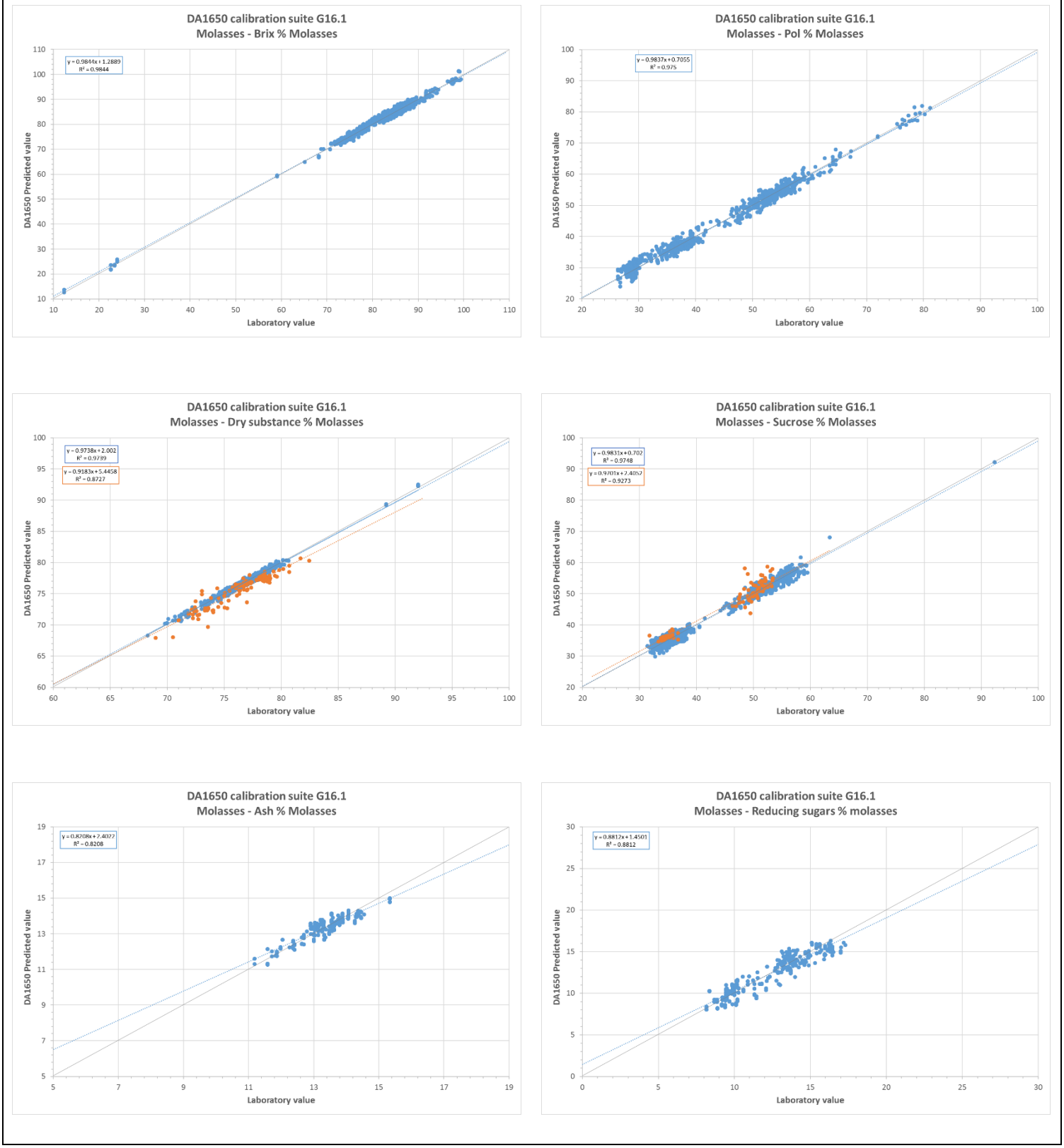

Figure 128: Calibration and validation (where available) plots of the G16.1 calibration suite for molasses

For each product, the calibration statistics for the G16.1 models far surpassed those for the R G16.1 models. On all occasions, the standard error of calibration (SEC) was lower and the coefficient of determination (R^2) was higher for the G16.1 models. Similarly, the validation statistics show that the predictive performance of the G16.1 models was much better than that of the R G16.1 models, as demonstrated by their low SEP values. These figures are supported by the calibration /validation plots provided in Figure 127 and Figure 128. All of the R G16.1 calibrations show significantly more scatter about the regression line and in the cases of pol and sucrose, there is little trend visible at all. The validation data for both models has not been subjected to any outlier testing. All models show slightly more scatter than would be optimal, but once again, the scatter for the R G16.1 models show little relationship between the NIR predicted values and the wet chemistry values. With the data available, it would be expected that the predictive performance of the models without validation would be equally poor.

Selection of specific wavelengths were expected to improve the predictive performance of the models by targeting only the relevant wavelengths in the spectrum. To evaluate how effective this process was, the coefficient plots for each analyte model were compared. The coefficient plot essentially identifies the areas of the spectrum that contribute most to the structure of the model. In a pharmaceutical application where the matrices are a lot less complex, the loadings plot typically matches the spectrum of the target analyte. This is rarely the case in agriculture due to the highly variable matrix, which contains large numbers of chemical constituents.

Plots of the calibration coefficients for the two model suites are provided for sucrose (Figure 129), pol (Figure 130), brix (Figure 131), dry substance (Figure 132), ash (Figure 133) and reducing sugars (Figure 134). Regions of significance in a coefficient plot are apparent by their deviation, either positive or negative, from the zero point, or centre point. If the selected wavelength regions were truly appropriate, they would be of similar shape and relative amplitude to those seen in the G16.1 models. Additionally, all vibrations over a certain amplitude would be included. It is clear in these figures that this is not the case. Some, such as ash (Figure 133), show little similarity in either shape or amplitude, whereas others, such as reducing sugars (Figure 134), show similarities between the two plots, but experience the occasional inversion of the signal. Inversion of the signal is not unexpected. The reduced number of variables means the model needs to explain the distribution of samples with less information. Wavelengths that were relatively minor contributors to this division when the full spectrum is included can easily become the most significant, of the small portion of the spectrum selected for the reduced calibration model.

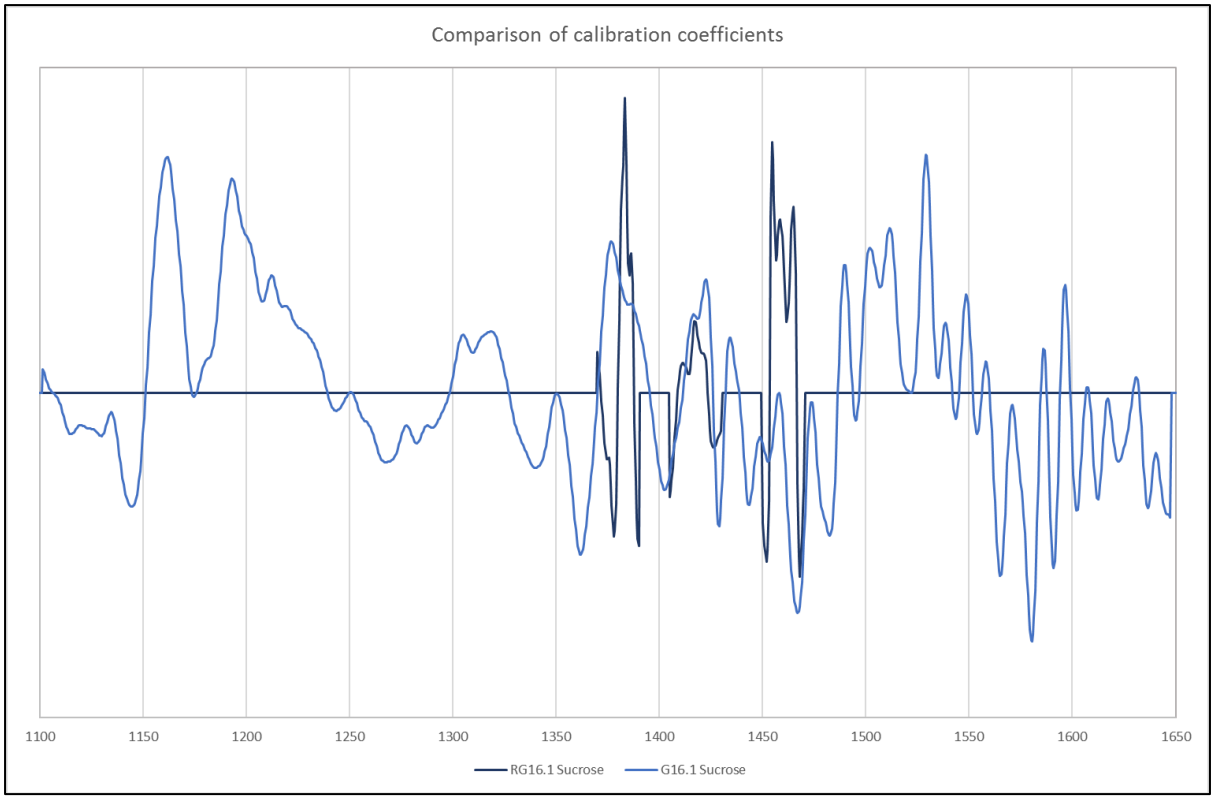

Figure 129: Comparison of G16.1 and R G16.1 calibration coefficients for molasses sucrose

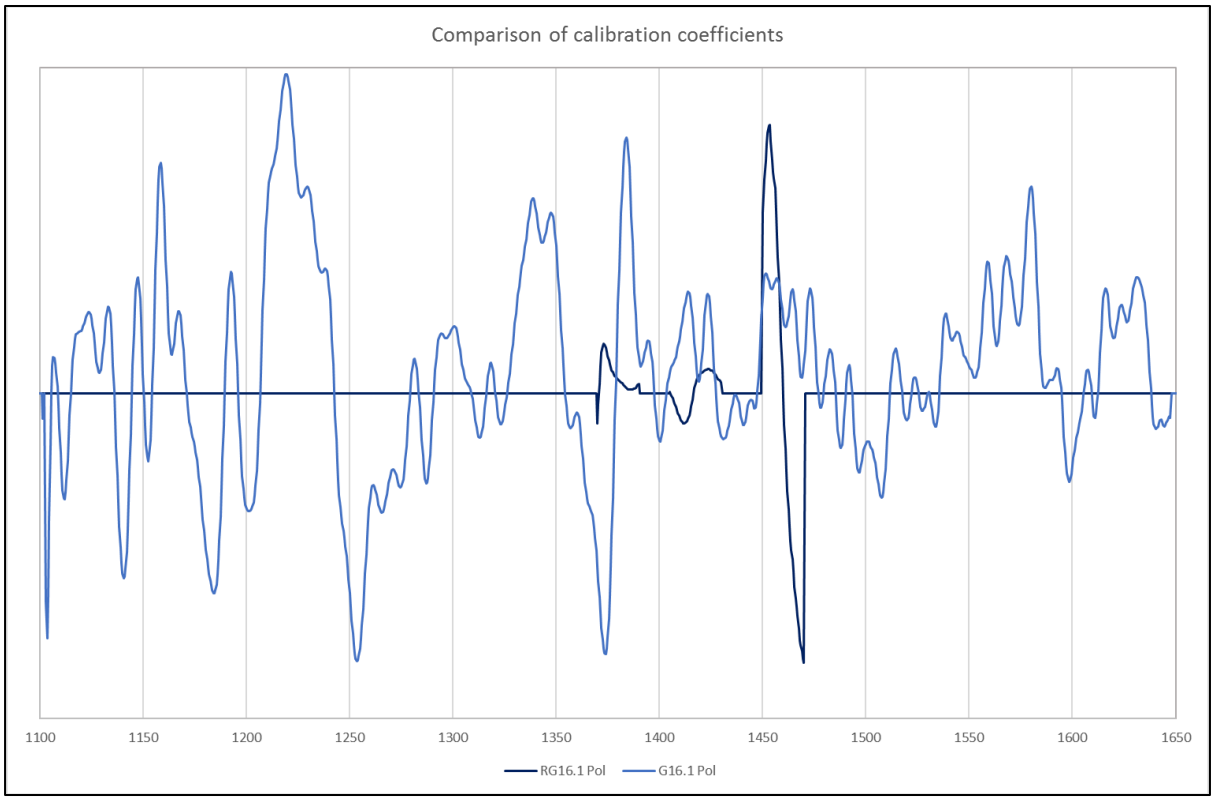

Figure 130: Comparison of G16.1 and R G16.1 calibration coefficients for molasses pol

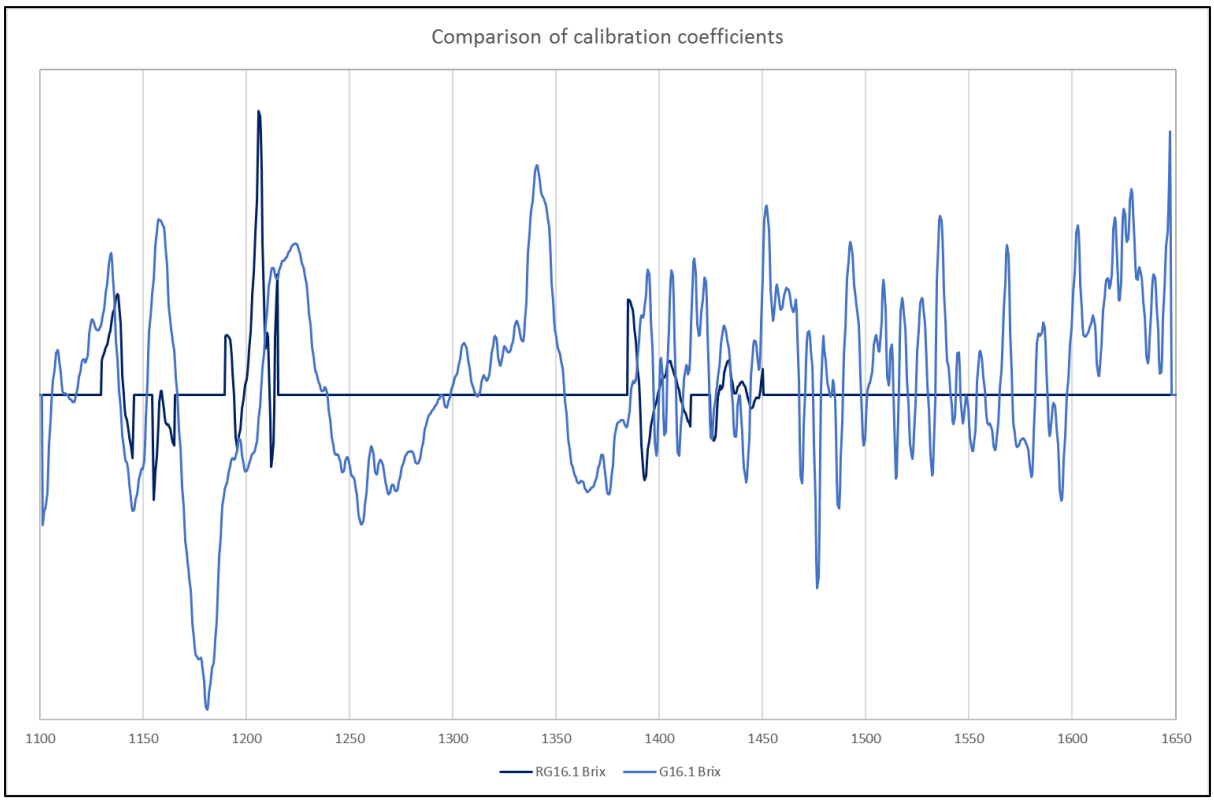

Figure 131: Comparison of G16.1 and R G16.1 calibration coefficients for molasses brix

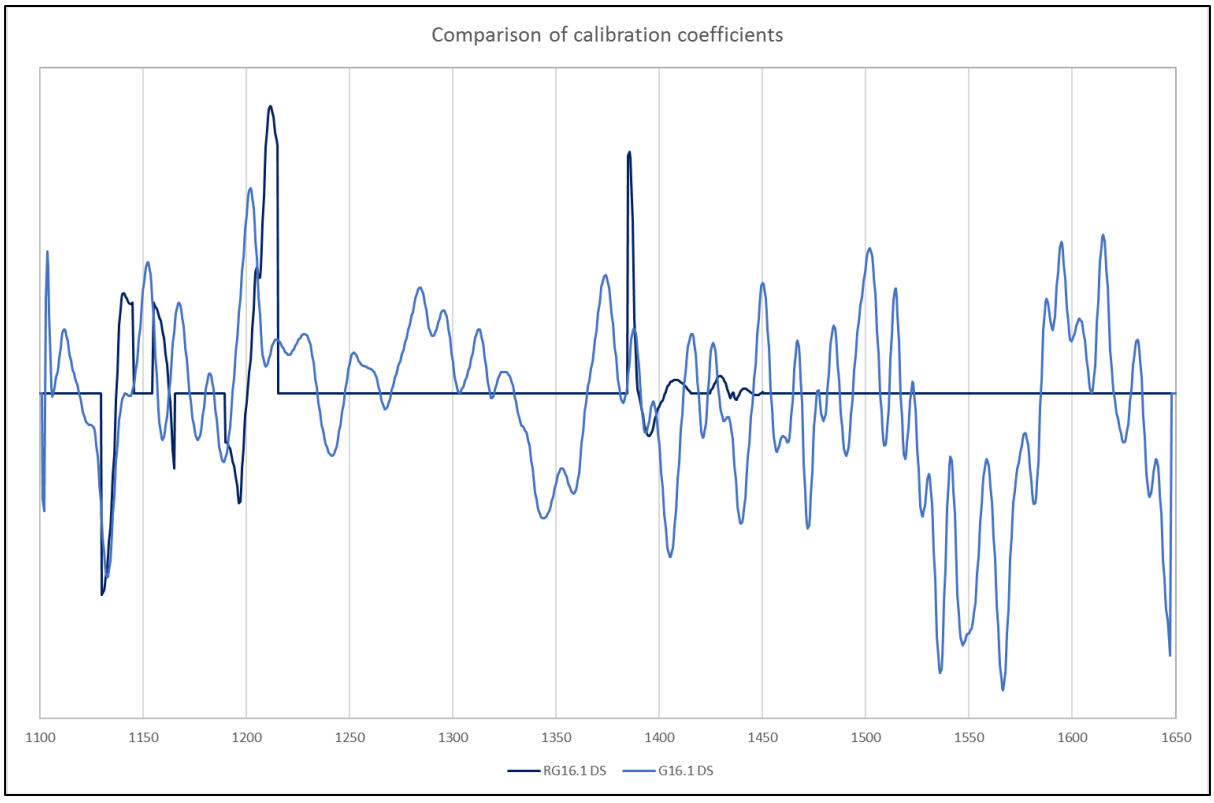

Figure 132: Comparison of G16.1 and R G16.1 calibration coefficients for molasses dry substance

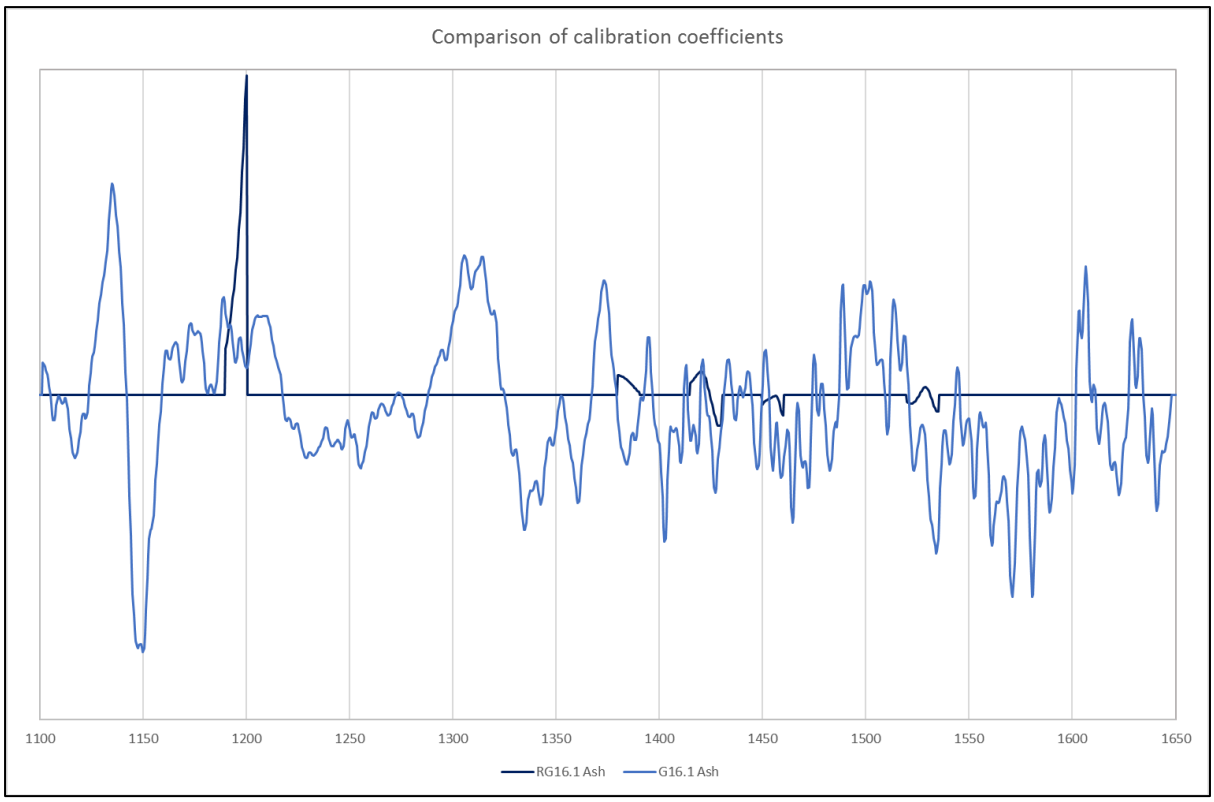

Figure 133: Comparison of G16.1 and R G16.1 calibration coefficients for molasses ash

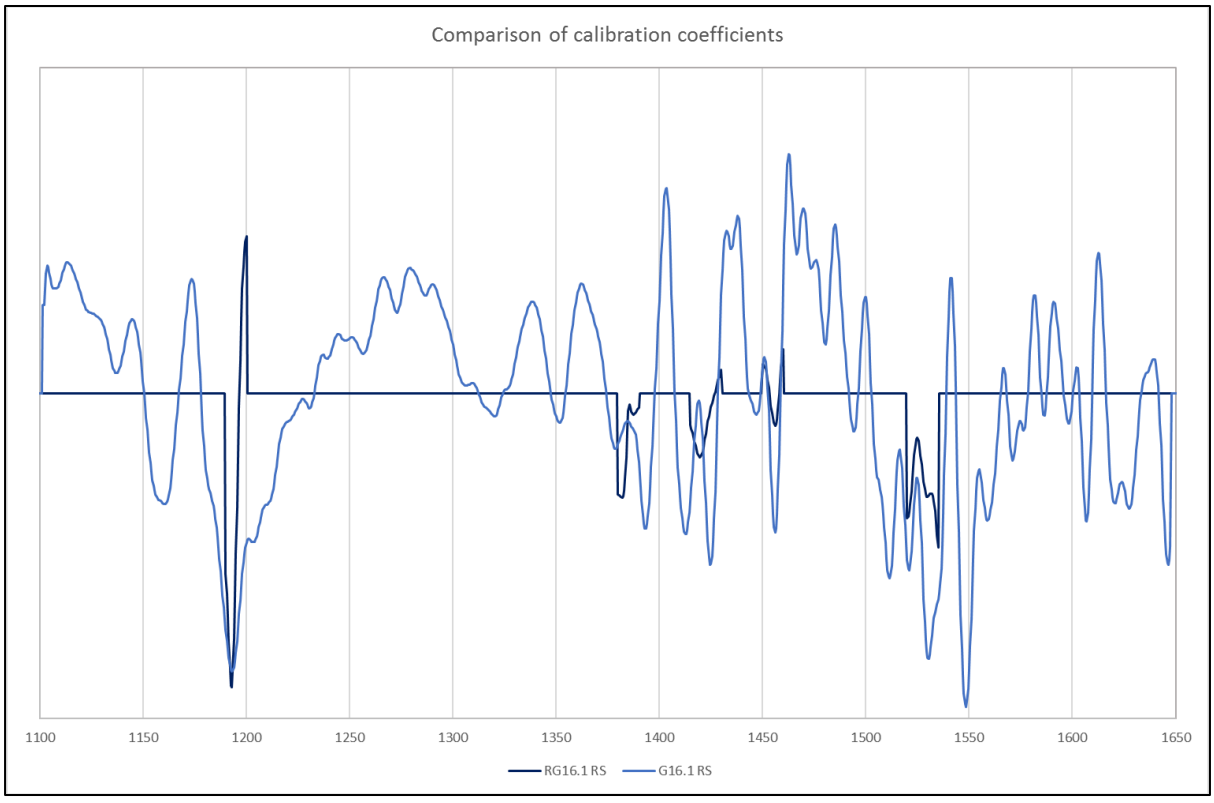

Figure 134: Comparison of G16.1 and R G16.1 calibration coefficients for molasses ash

The relatively poor matching of the R G16.1 calibration coefficients to the G16.1 calibration coefficients and the predictive performance of the models indicates that the method used to select key variables, or wavelength regions, was not appropriate. This is due to the slight differences in the PCA and partial least squares regression (PLS) algorithms. PCA uses all variables in the X-matrix (in this case, wet chemistry, NIR, IR and TGA data) equally to map variance in those variables (Martens and Næs, 1992). Essentially, it simply probes for collinearity within a dataset. PLS, on the other hand, investigates the variance in the X-matrix (in this case the NIR spectra) as an explanation of the variance in a Y-matrix (in this case the wet chemistry analyte) (Martens and Næs, 1992). Considering this, the first PC of a PCA describes the maximum variance of the X-matrix, whereas, the first factor of PLS describes the maximum variance of the X-matrix that describes the variance in the Y-matrix, which will be a percentage of the overall variance in X, and potentially a small one. The variance explained by the coefficient plot (which is a combination of the loadings of the first n factors) will be captured in the loadings of the PCA, but it may be in a later PC, or be split among multiple PCs. The PCA analyses investigated for this work probed only the variance attributed to the first two PCs, which combined, explained approximately 75 % (Figure 95) and 90 % (Figure 106) of the variance in the data. PLS is more likely to extract and amplify small variances in X-matrix that are specifically related to variance in the Y-matrix and discard gross spectral variation at specific wavelengths that are contributed to by multiple analytes.

What this tells us in a practical sense is that our highly complex agricultural samples produce spectra where each peak represents stretching and bending vibrations from multiple analytes. The larger dataset provided by a full wavelength range allows the collinearity of the spectra to account for this by using a combination of positive and negative responses to provide a ‘variance fingerprint’ (correlation coefficient) for the analyte. The high level of response across the whole spectral region demonstrates this.

This analysis has indicated that further investigation of spectral targeting is not worthwhile in this manner, as the spectral response to the complex samples is near impossible to interpret. Alternatively, the correlation coefficients may be interrogated to identify which regions of the spectrum are active in the calibration model, that can be tracked back to proposed band assignments in the NIR spectrum; this may give some insight into whether the calibration is modelling a direct chemical response or a co-correlation.

The coefficient plots for molasses all show the majority of the strong chemical response is between 1100 - 1400 nm, which is equivalent to 9091 - 7143 cm^{-1}. This mostly captures the second overtone CH stretching at the higher frequencies, and the CH combination bands in the lower frequencies. There are three potential water vibrations present in this region also: $v_1+ v_2 +v_3$ water combination at 1135 nm, $v_1+ v_2 +v_4$ water combination at 1345 nm and the anti-symmetric and symmetric OH stretch water combination at 1378 nm. The correlation coefficients for each analyte are different, which indicates that the models are different and not simply providing the same prediction with a scaling factor. Surprisingly, there is a significant difference between the pol and sucrose models and the brix and dry substance models. This may be due to the effect of impurity loadings on the apparent purity reference methods.

Another technique exists that may facilitate improved and more specific models. It involves the use of multiplicative scatter correction (MSC) using a target analyte spectrum as the ‘average’ (2015). This is feasible when a pure spectrum of the target analyte can be obtained. For our application, this is possible for sucrose, but may be more challenging for analytes such as dry substance, pol, brix etc. However, this technique is not available in the software used to calibrate and run FOSS instrumentation and so would require some software development to implement on a DA1650.

6.2.3. Analysis of fresh raw sugar

In the last few years, it has become evident that the NIR spectroscopic analysis of raw sugars fresh from the belt failed to predict pol and moisture values accurately, despite success with older samples. This prompted further investigation, as rapid feedback of raw sugar is one of the most high value areas for laboratory NIR spectroscopic systems.

6.2.3.1. Time-delay analysis of fresh raw sugar

To investigate this further, trials were conducted at Mill 1 on a FOSS InfraXact™ (IX) and Mill 11 on a DA1650 system in the 2013 season. Brand 1 sugar samples were collected from the sugar belt, immediately after exiting the sugar drier and the age was carefully monitored with a timer. The samples were scanned on an NIR instrument periodically over a 24-hour period and the corresponding age recorded. This process was reported by Steve Staunton (Staunton, 2014). These experiments identified that the change in predicted value of pol was consistent and repeatable between mills and instrument types. The trend was logarithmic and variation of the predicted value outside of the error control limit (1.2 x standard error of cross validation) continued until 180 minutes, although the majority of the change occurred within the first 50 minutes.

A correction algorithm was developed by applying a linear regression to the normalised data between 20 and 180 minutes (after the rapid change in variation had finished). This correction was provided to the mills as an excel worksheet, where the user could enter the age of the sample and the predicted pol value and a corrected value would be returned. This was limited to a sample over 20 minutes old. Younger samples could be corrected, however a logarithmic adjustment would be required and small misjudgement in the age of the sample could cause large adjustment errors.

The cause of the change in predicted value of pol in fresh raw sugar was unknown, but the preliminary data suggests that there is a change in the reflectivity of the sample as it ages. This could be caused by many factors, including: temperature, Maillard reactions, oxidation or other physical factors.

Designing experiments to test the cause of the variation in the milling environment was challenging due to the limited resources available and the rapid time-frame required for the analysis to take place. Realistically, temperature was the only controllable variable. However, this has the potential to control the rate of reactions, which could guide evaluation of potential cause.

A trial to collect time-delay data on two brands of raw sugar was completed at Mill 11 in the 2015 season to supplement the existing data from Mill 1 and Mill 11 in the previous seasons.

Each experiment typically ran for 80 minutes, which was not long enough for samples to fully stabilise to within ECL, as reported previously. Regardless, each experiment requires the data to be normalised so the results can be compared. The last data point for each experiment will have the least variability from the ‘aged’ predicted value. This was used as the control value. The datasets were normalised by subtracting the final predicted pol from the predicted pol value at each of the time points.

The normalised pol results for each experiment were plotted against the age of each sample (Figure 135). The relationships between normalised pol and time at all temperatures and for both sugar types were logarithmic, as previously reported. The degree of the logarithmic curve does vary between replicates and experiments but is not dependent on temperature. Similarly, the normalised moisture against age shows the same relationship (Figure 136). This indicates that the change in predicted pol and moisture values are not a function of temperature.

These plots also suggest the change is not due to a chemical reaction occurring in the sugar, as this would be catalysed by the change in temperature; at higher temperatures the rate of the initial change would be faster and the samples would stabilise earlier.

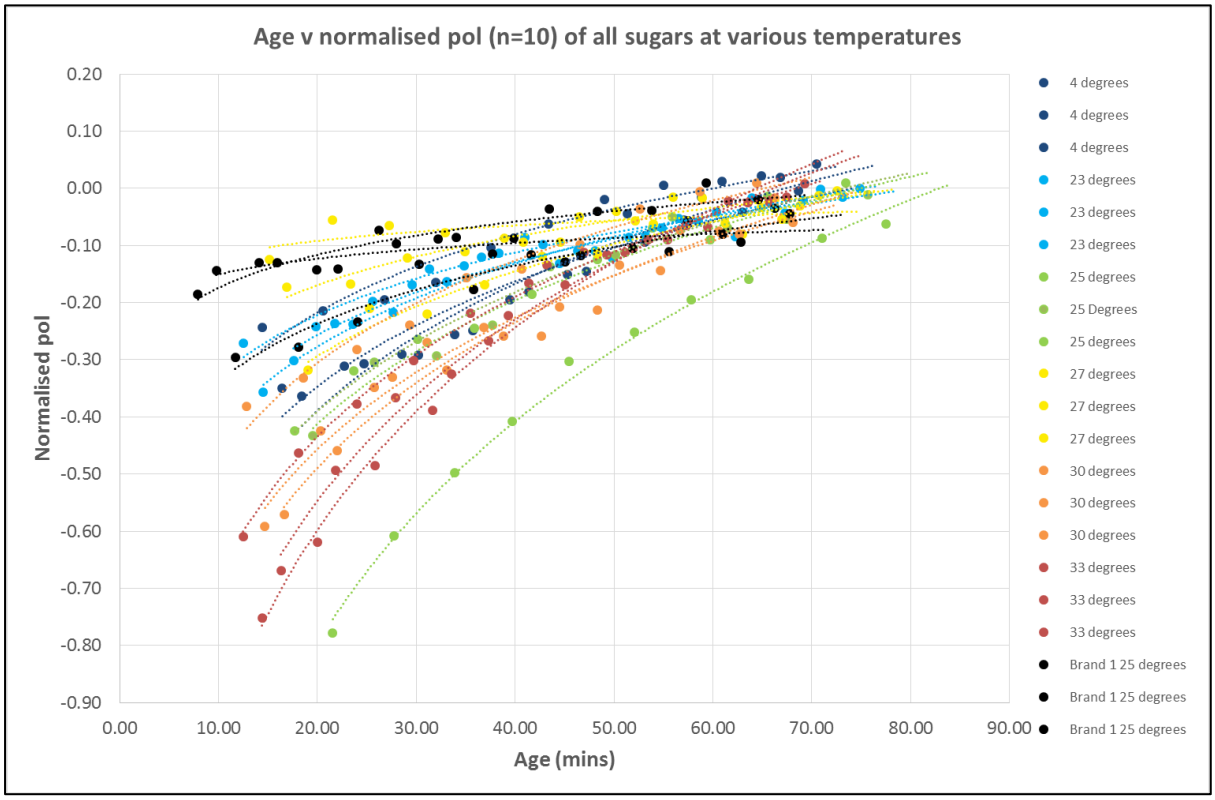

Figure 135: Age v normalised pol of sugars at various temperatures

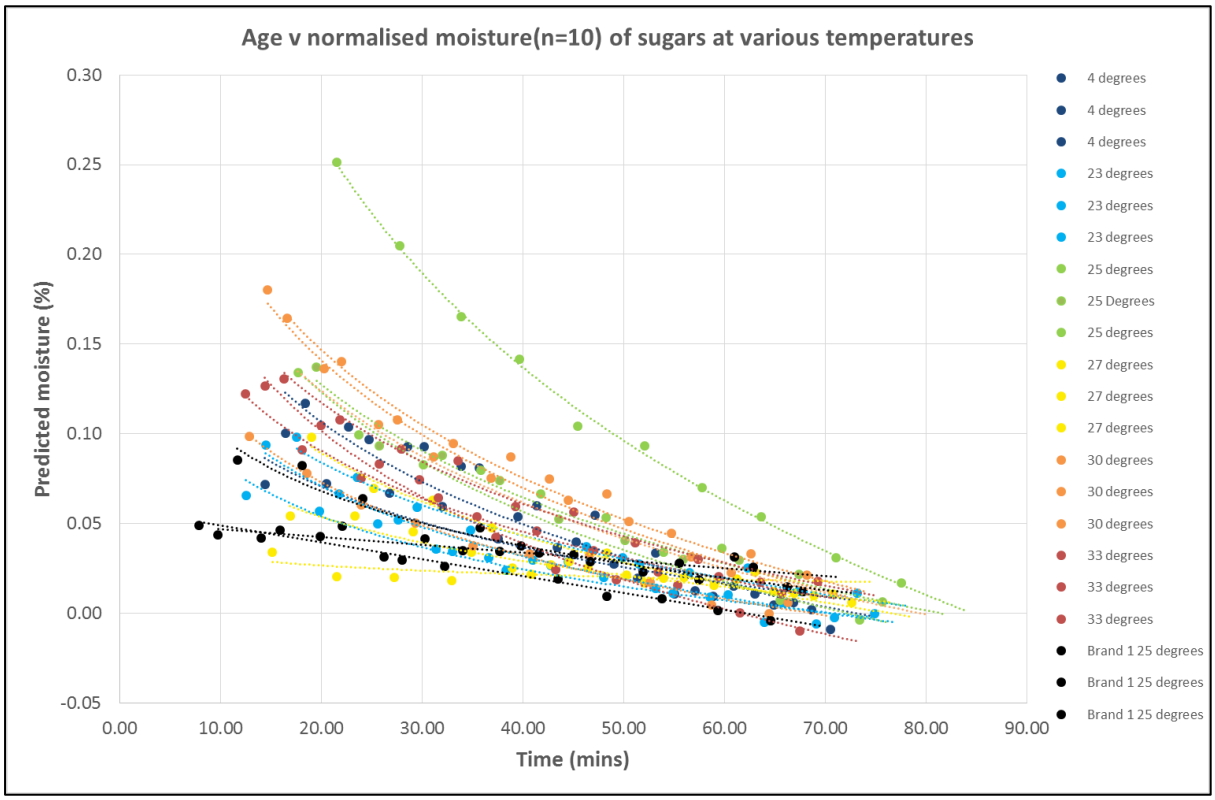

Figure 136: Age v normalised moisture of sugars at various temperatures

This is supported by the plots of instantaneous rate of change in pol (Figure 137) and moisture (Figure 138) v age, which again do not trend with temperature.

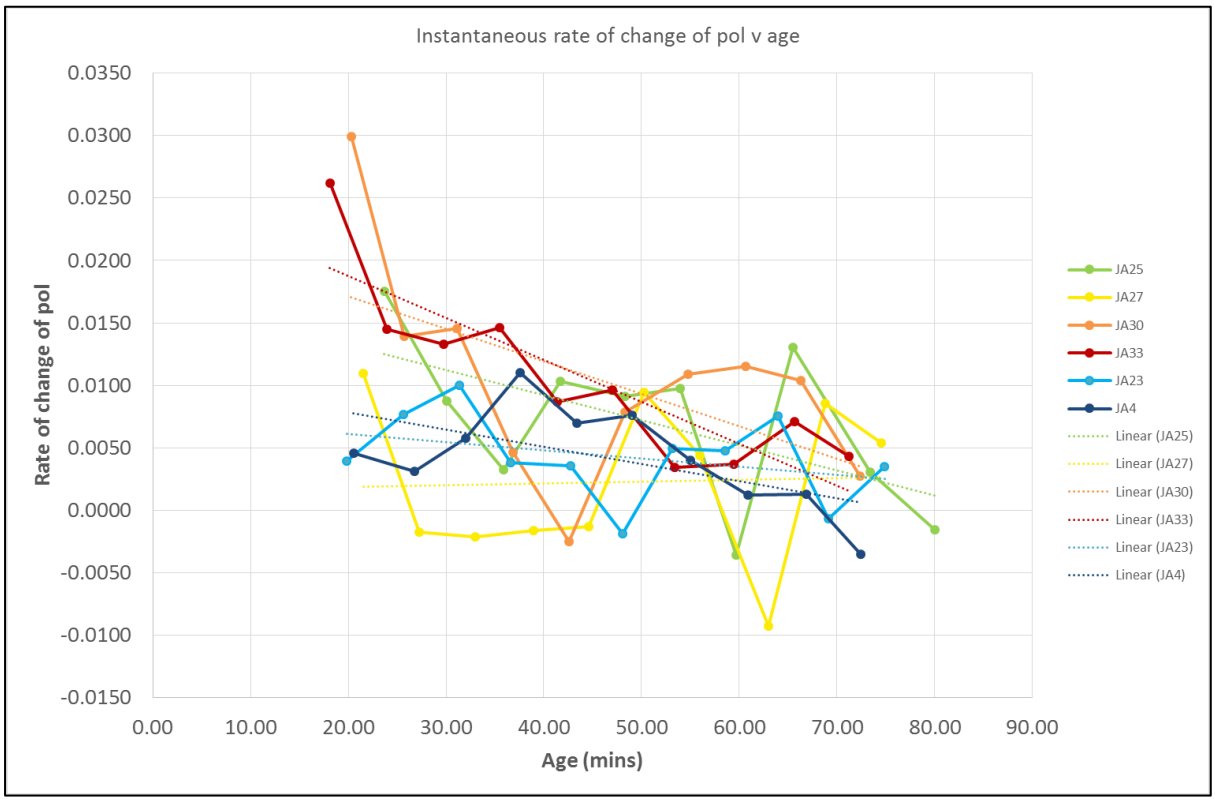

Figure 137: Instantaneous rate of change of pol by age

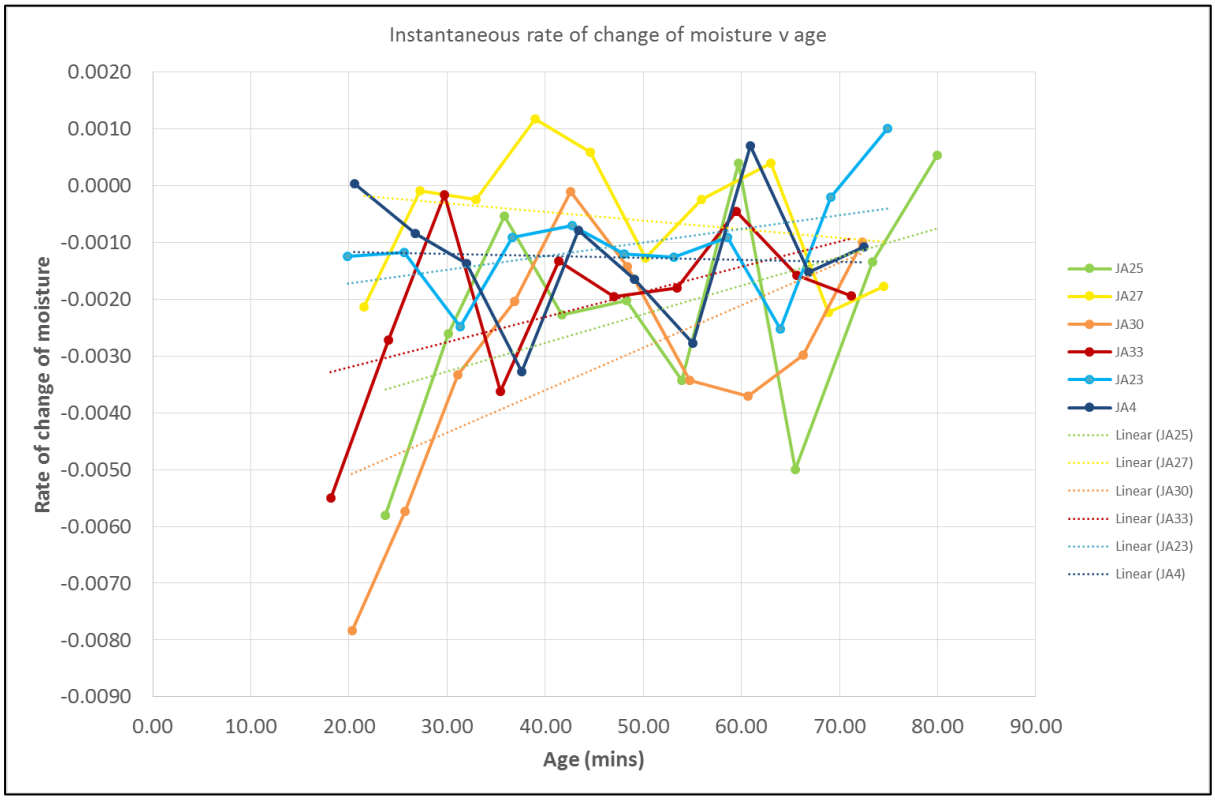

Figure 138: Instantaneous rate of change of moisture by age

Finally, the variability and scatter observed when the normalised pol and moisture are plotted against temperature (Figure 139 and Figure 140), confirms that the change in the predicted values are not due to temperature or chemical changes.

A third potential cause for the change in reflectivity of the sample was oxidation. To test this, samples were stored under vacuum between analyses to create a low oxygen environment. If the change is due to oxidation, the change in predicted pol values with time will be slower with the vacuum treatment. A plot of the normalised pol v age for the samples treated under vacuum showed interesting results (Figure 141). The relationship for the three replicates is linear. Otherwise, there does not appear to be a change in the rate of the predicted values compared to the normal samples, as a result of the vacuum. This indicates the change is not due to an oxidative reaction.

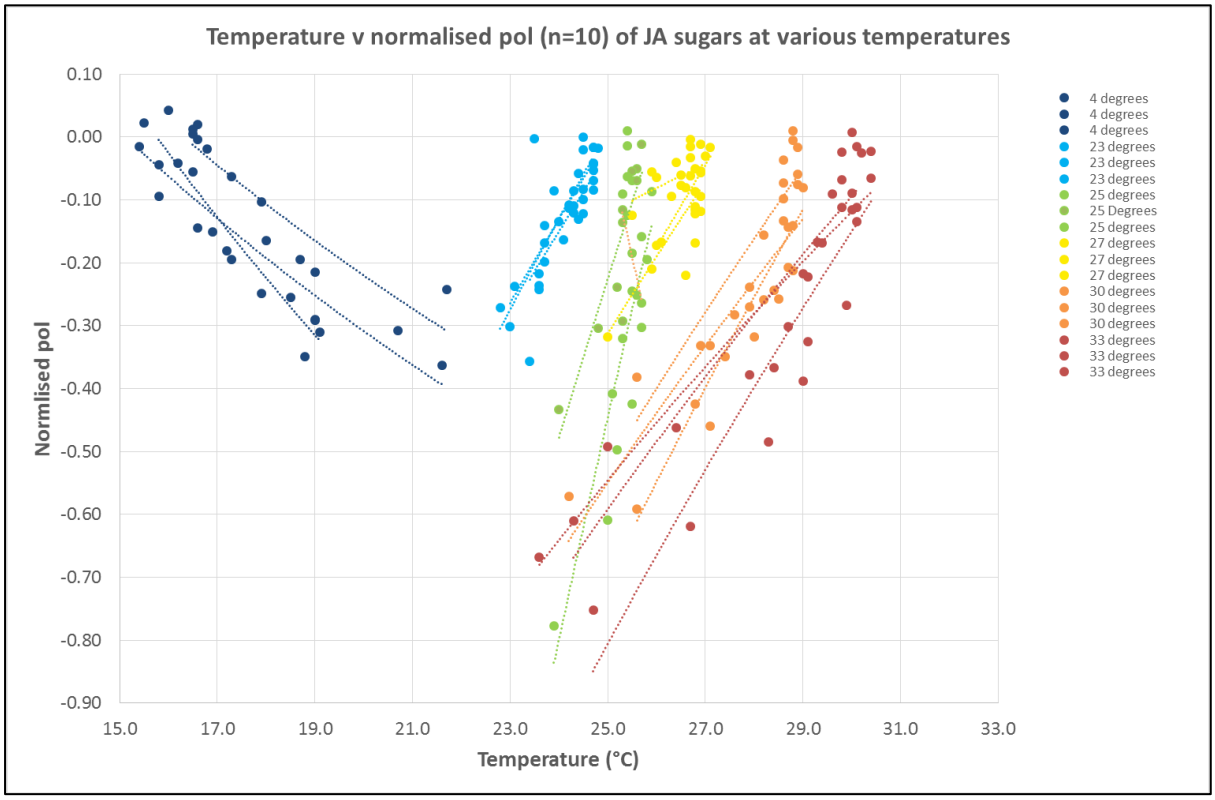

Figure 139: Normalised pol v temperature

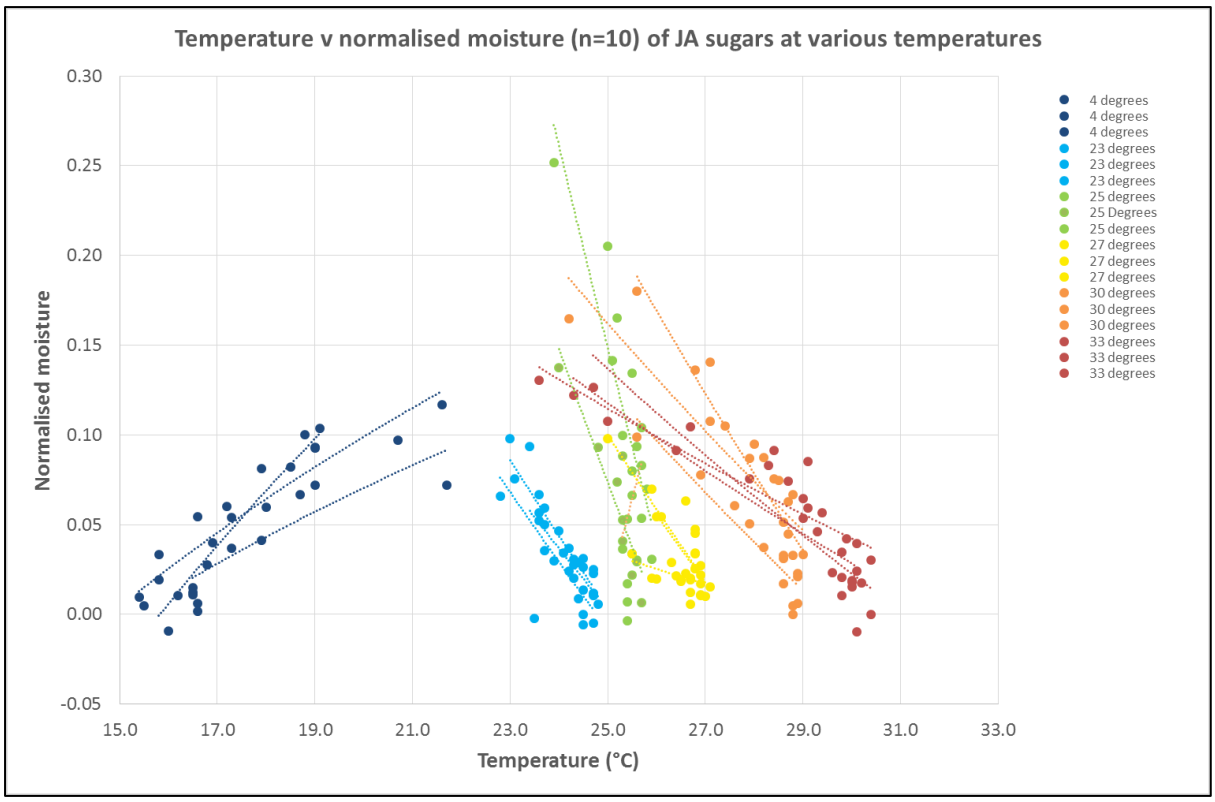

Figure 140: Normalised moisture v temperature

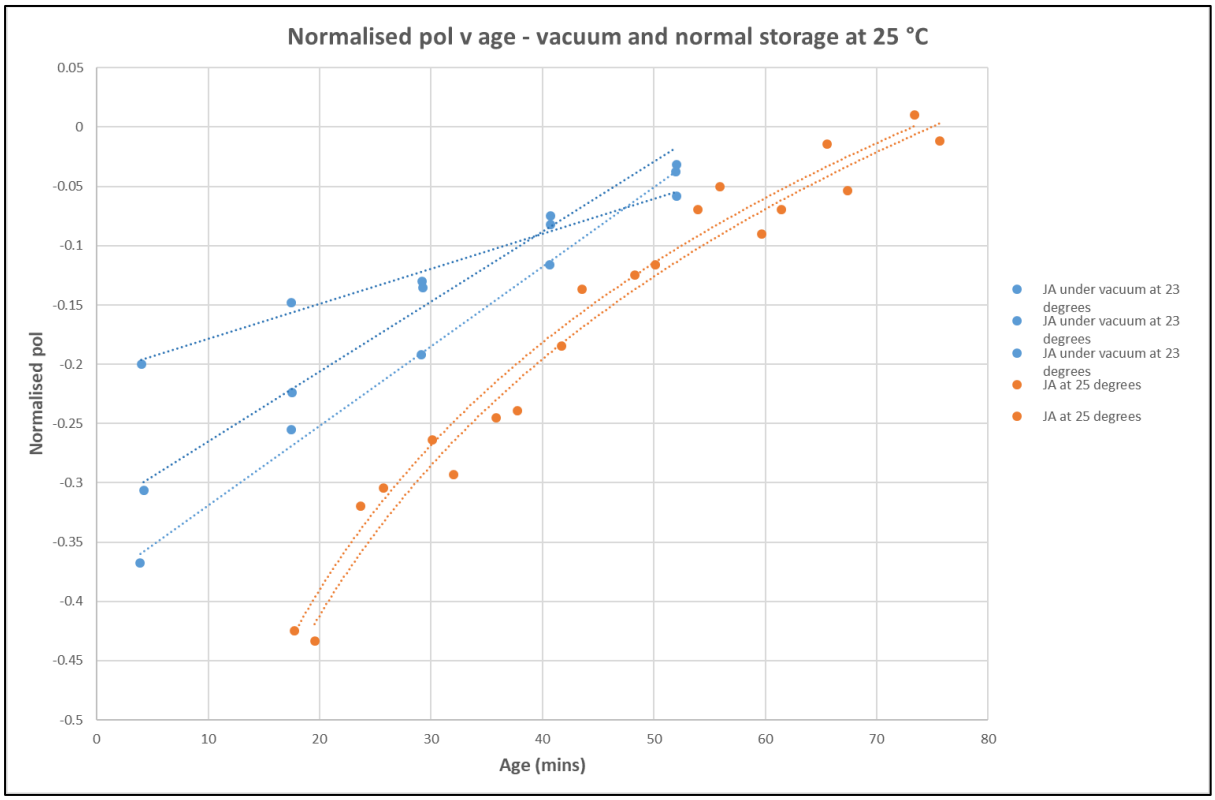

Figure 141: Normalised pol v age for vacuum and standard pressure samples

So far, the experimental data has indicated the change in predicted pol and moisture values is independent of temperature, chemical reaction and oxidation.

Despite this, something is causing a repeatable change in reflectivity of the sample. It's proposed that this may be due to moisture in the sample as the logarithmic change in predicted pol value with time is indicative of a desorption isotherm.

There are three types of moisture in sugar: free water, which is that accumulated in the syrup film and easily removed upon drying; bonded moisture, which is trapped in the amorphous layer of the crystal; and inherent moisture, which is trapped in the crystalline lattice of the sugar and hard to remove.

Migration of moisture in sugar is a well-recognised phenomenon and is responsible for the need to condition sugar to prevent caking and deliquescence ((Chen and Chou, 1993)). Migration typically occurs in three ways, depending on the surrounding environment. First, is the migration of bonded moisture into the molasses layer, second is the migration of moisture from the atmosphere into the molasses layer of the crystal and finally, supersaturated molasses releasing moisture from the sugar as it crystallises. Potentially, a combination of each of these effects takes place until an equilibrium across the crystal-molasses layer and immediate environment are reached.

This curing of the sugar changes the appearance of the sugar, which start as glassy crystals that become dull over time. The curing or conditioning of sugar is reported to take approximately three days and none of the literature mention rapid changes in the first hour of exiting the dryer.

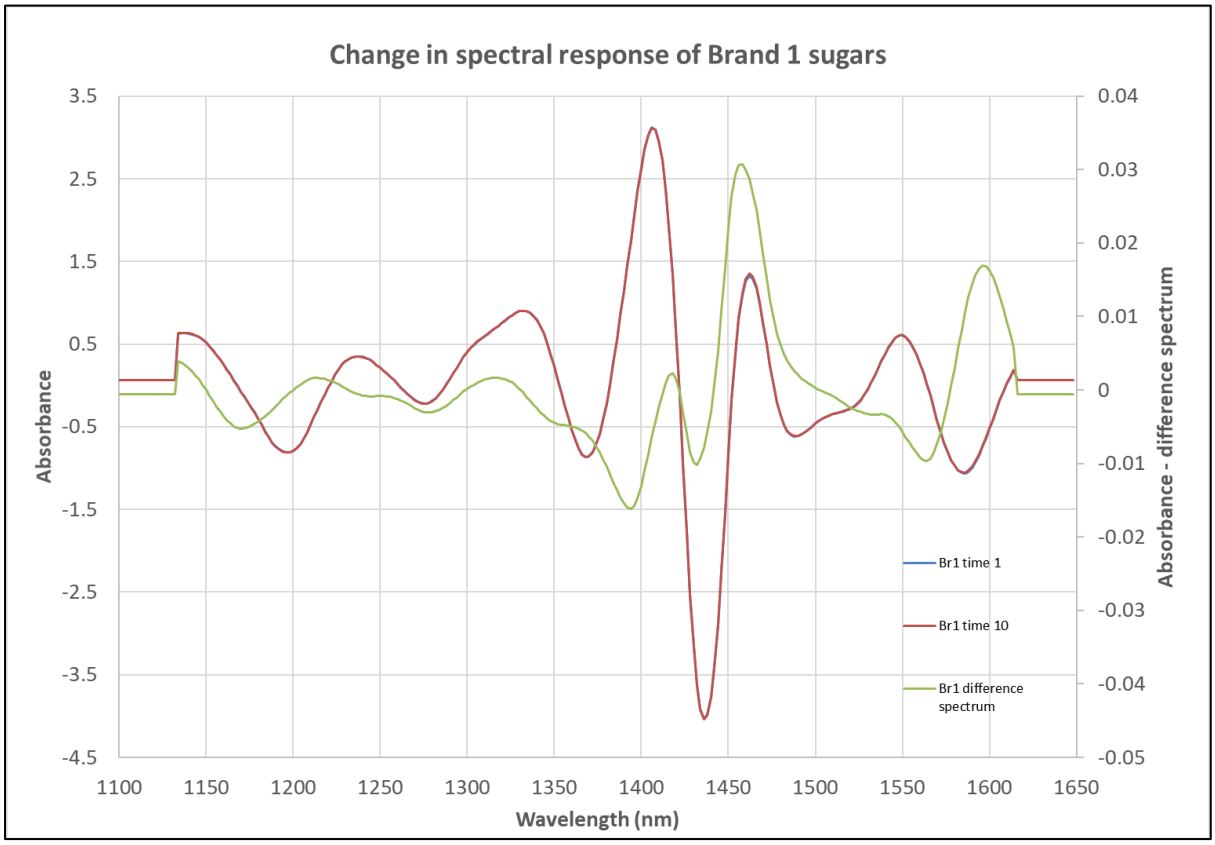

Figure 142: Change in spectral response of Brand 1 sugar (blue behind red)

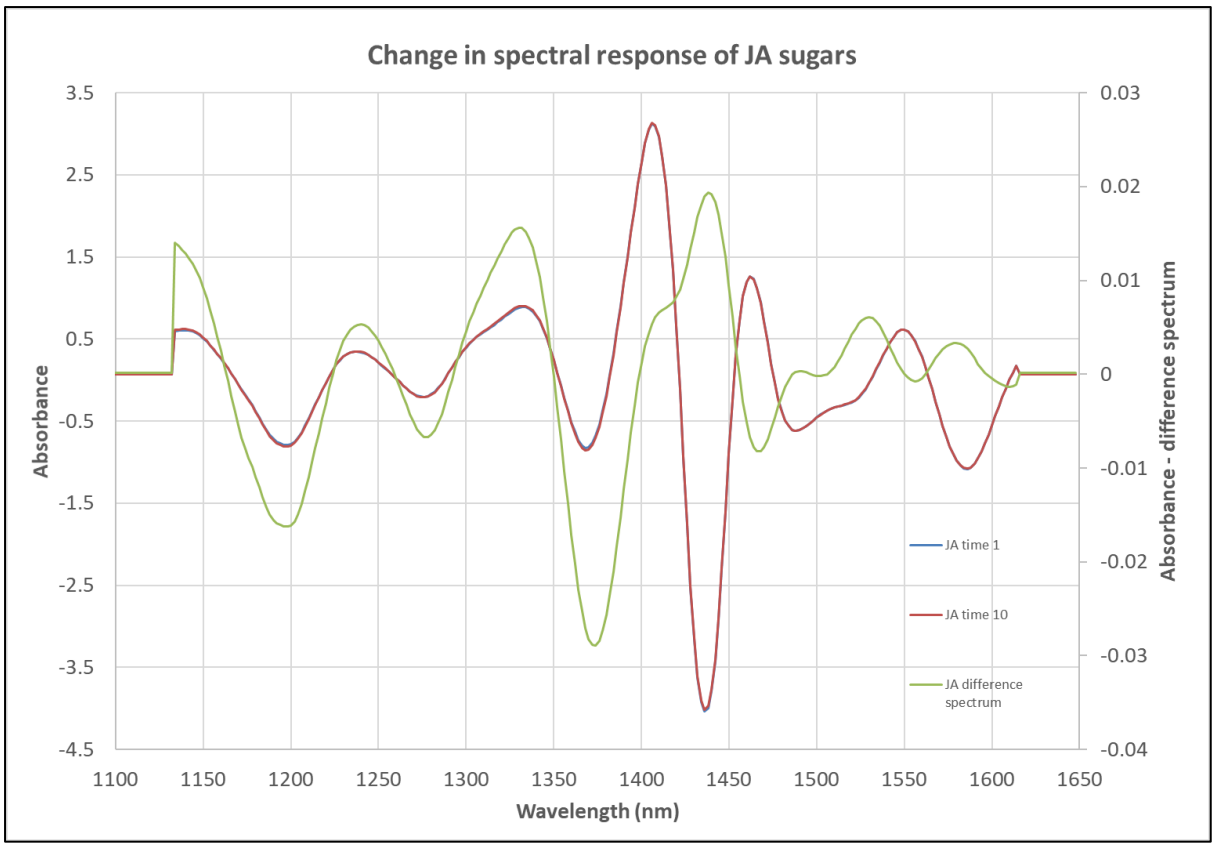

Figure 143: Change in spectral response of JA sugar (blue behind red)

Figure 142 and Figure 143 show the change in spectral response with time for Brand 1 and JA sugars. This shows that the change that is occurring is not the same for the two types of sample, which is unexpected. In particular, is the variability between 1350-1650nm. This includes the moisture region at around 1450 nm.

More detailed investigations of the spectral change with time are provided in Figure 144 and Figure 145. Similarly to Figure 142 and Figure 143, there is considerable difference between the two brands. Brand 1 shows a lot more variability across the whole spectrum, but does show trending from young to old (or vice versa) for some peaks, likely to be ascribed to water. Alternatively, the JA spectra are a lot more organised, particularly in the region below 1400 nm.

Both plots show a transition point at around time point 5, where the spectra change markedly, and do not show a simple increase or decrease of a specific peak. An example of this is between 1400 - 480 nm of Figure 145. This may represent two different moisture migration processes occurring in succession.

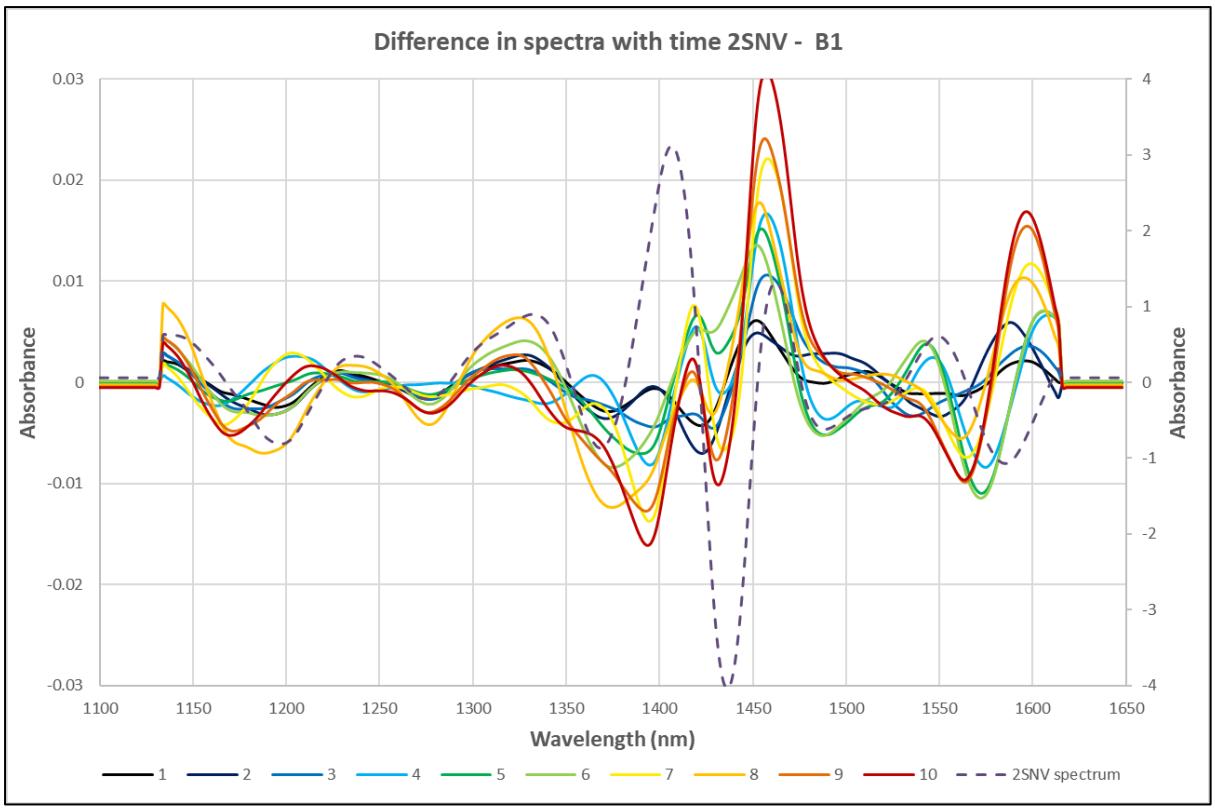

Figure 144: Difference in spectra with time - Brand 1

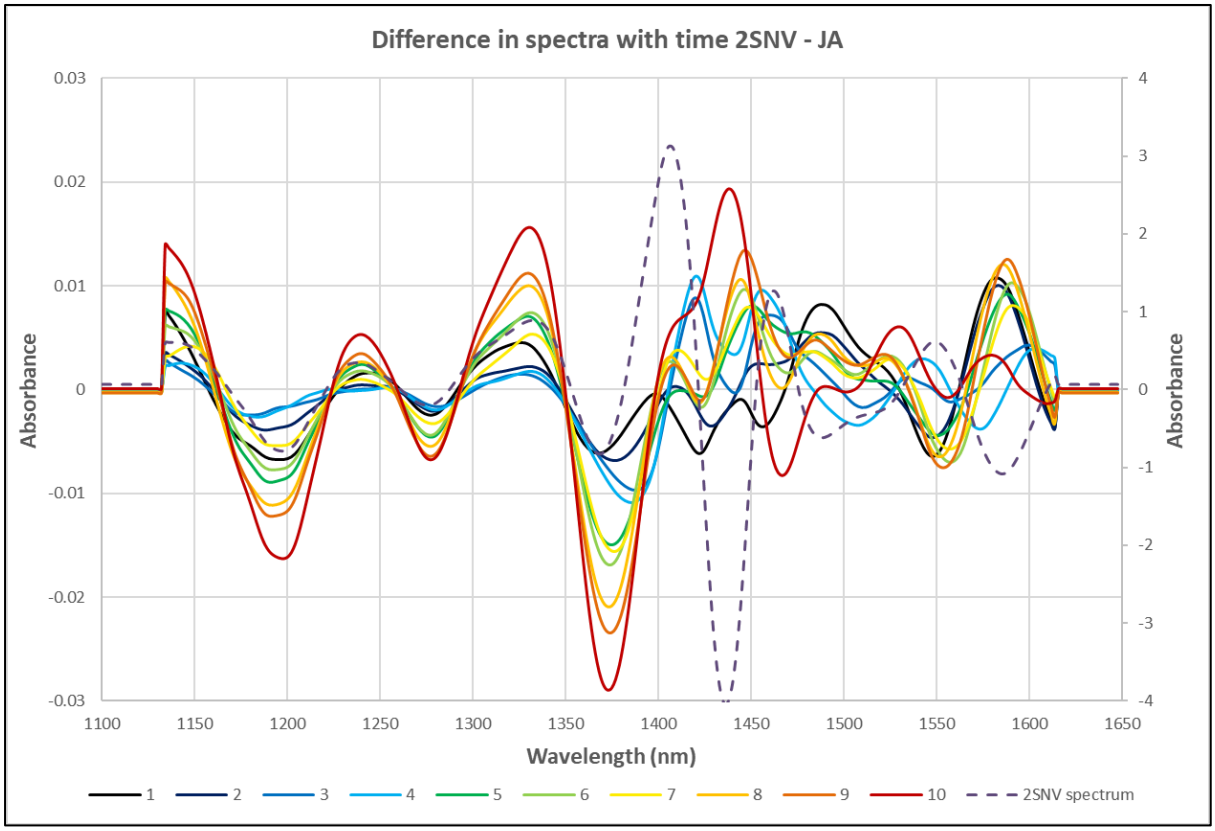

Figure 145: Difference in spectra with time – JA

The linear relationship observed in Figure 141 may also support the theory that the change in spectral response is due to moisture migration in the sample. By holding the sample under vacuum, the vapour pressure of water is lowered, forcing removal of moisture from the sample. This would constantly force movement of moisture from the molasses layer followed by bonded moisture in the crystal. Alternatively, holding the sample under vacuum may have prevented exposure to a high humidity environment, preventing absorption of moisture into the molasses layer around the crystal. The complex logarithmic change in the results may indicate multiple moisture conditions changing within the sugar. Completing a similar time-based experiment but maintaining the sugar in a moisture-free environment would assist in confirming this theory.

Despite the testing indicating the change in pol with time is likely due to moisture migration through the crystal, it is probable that temperature is also making a minor contribution. It is known that temperature affects the predictive performance of NIR spectroscopy and the current calibrations have not yet been desensitised to temperature.

As the change in pol with time is most likely due to moisture migration and not oxidation or other chemical effect, molasses is no longer expected to exhibit the same trend. Despite this, a series of molasses experiments were performed and the results are shown in Figure 146. The plot shows repeated experiments of pol predictions with age. The variation in pol is within the standard error of calibration (Figure 146), which is the best possible error the calibration could produce for these values.

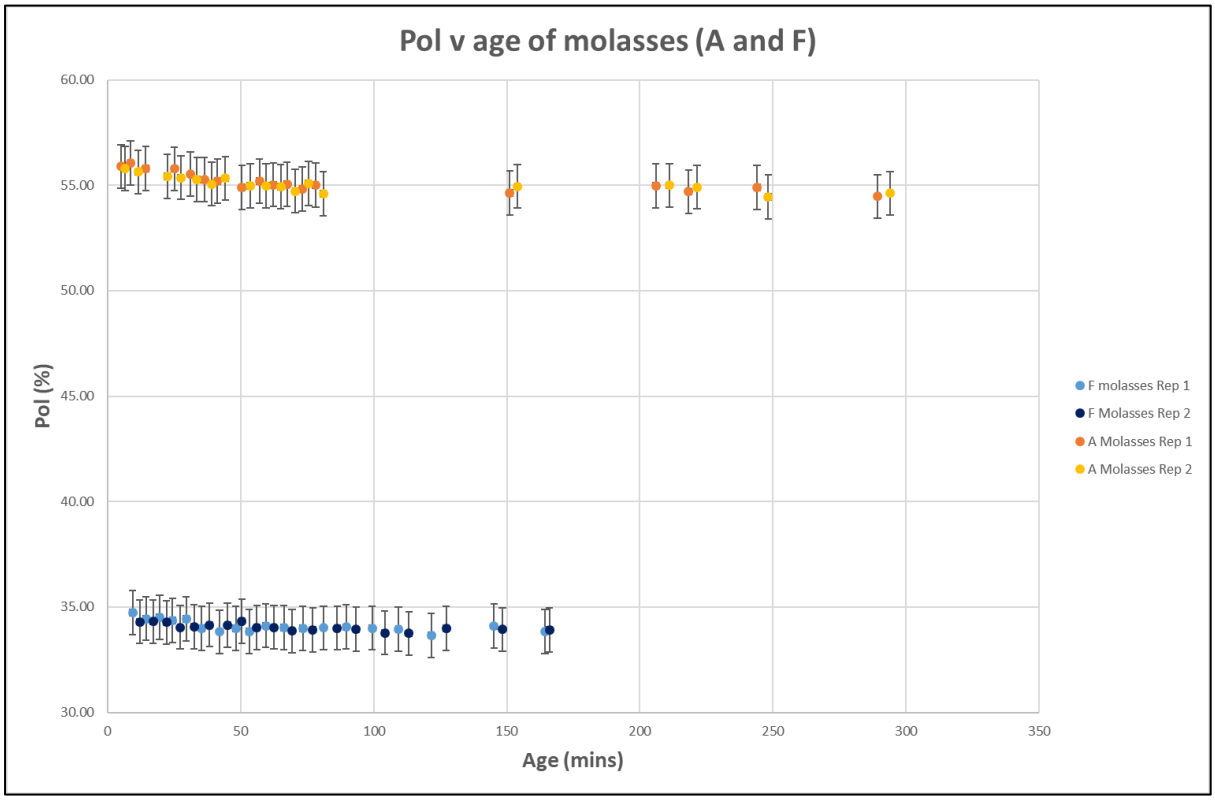

Figure 146: Pol v age of A and F molasses

6.2.3.2. Modelling fresh raw sugar

The development of a calibration model that is desensitised to the shift in NIR spectral response with time allows for an automatic adjustment and analysis of samples at any age. This provides a more useable solution than a post-process using a manual linear correction algorithm.

The desensitisation approach is an adaption of that by Cao, who used a similar technique, to desensitise NIR spectroscopic models against temperature (Cao, 2013).

Individual samples were scanned many times as the sample aged to map the spectral changes. The stabilised predicted values were assigned to all of the spectra in each sample set as reference values. This was combined with a subset of the G16.1 calibration set, identified for their unique spectral properties through a sample selection algorithm.

New calibrations were developed for moisture and pol using the standard calibration method. The calibration statistics are provided in Table 38 and the calibration plots are provided in Figure 147 and Figure 148.

Table 38: Calibration statistics for fresh raw sugar models

	SEC	R^2	Mean	Standard deviation	Min	Max	N	Factors
Pol %	0.091	0.99	-	-	92.36	99.86	507	12
Moisture %	0.026	0.94	-	-	0.08	1.06	502	10

SEC: standard error of calibration, R^2: coefficient of determination, N: number of calibration samples

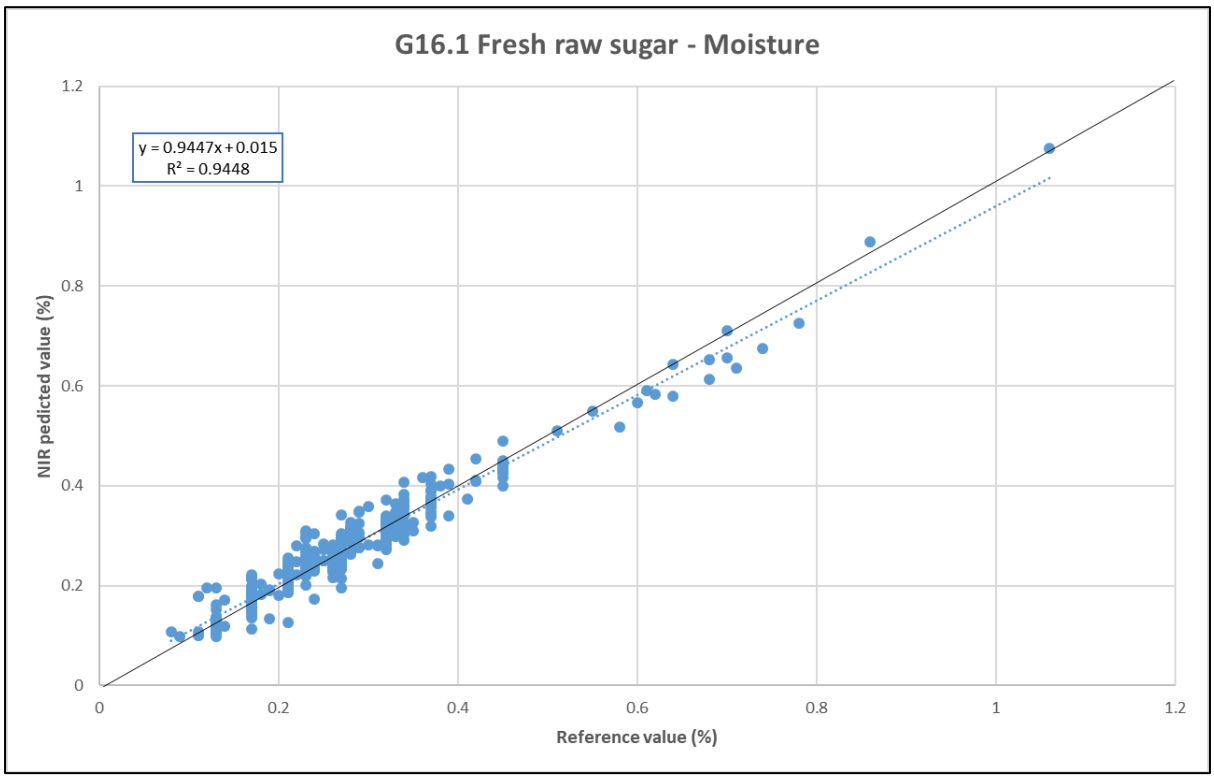

Figure 147: Calibration plot for fresh raw sugar moisture

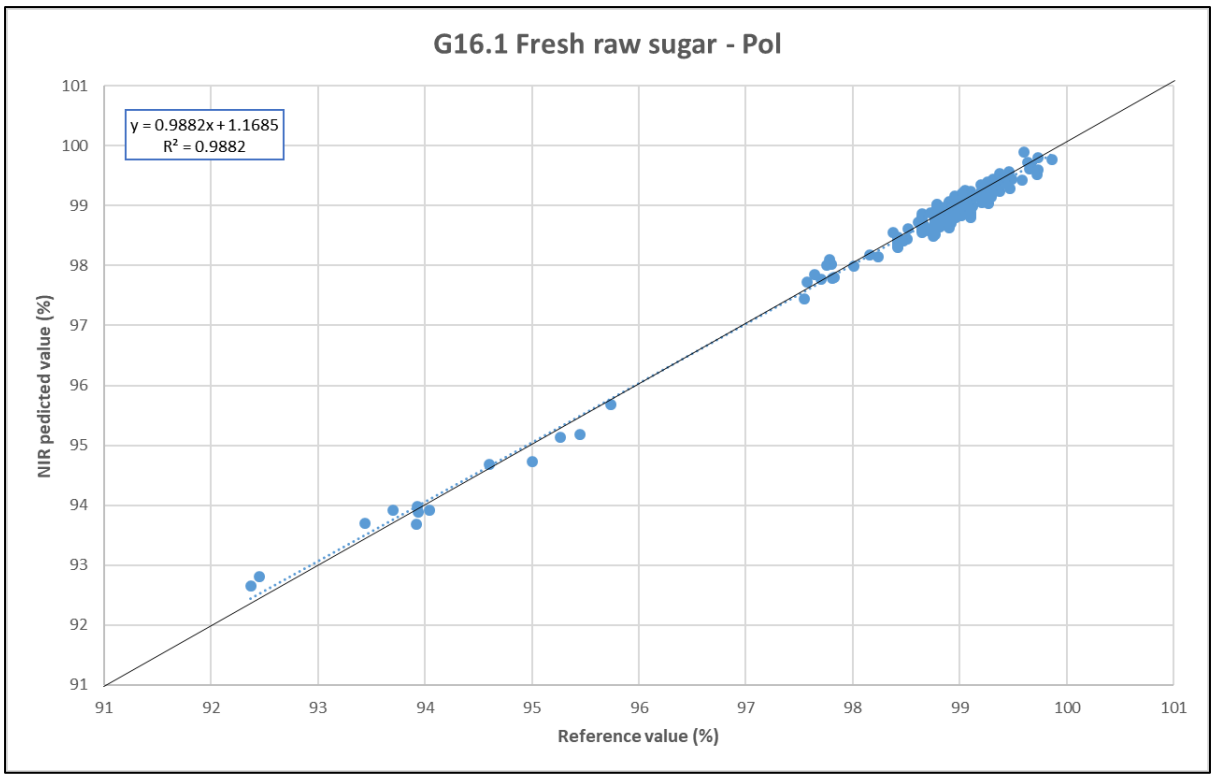

Figure 148: Calibration plot for fresh raw sugar pol

A set of independent validation time-delay samples were analysed by both the G16.1 Fresh models and the G16.1 models. In all cases, the predictions generated by the G16.1 Fresh models were consistent with time, rather than presenting as an asymptote (Figure 149). This is demonstrated for all validation samples by showing the variance (G16.1 - G16.1F) between the predicted values for the G16.1 and G16.1 Fresh models. Each sample, along with the bulk all show the characteristic asymptote shape, which demonstrates that the standard models under predict pol (Figure 150) and over predict moisture (Figure 151) when the sugar is fresh, but this is compensated for in the fresh models.

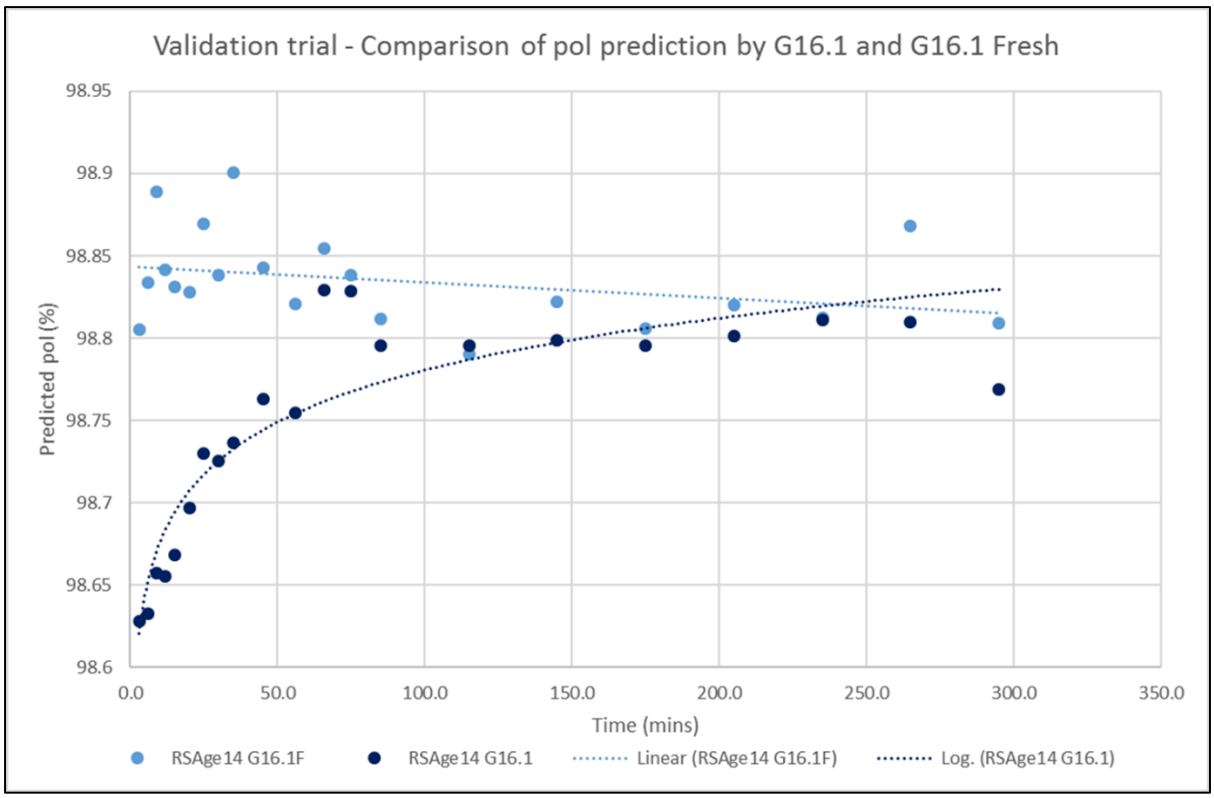

Figure 149: Comparison of the fresh raw sugar sample predictions generated by the G16.1 model and the G16.1 fresh models

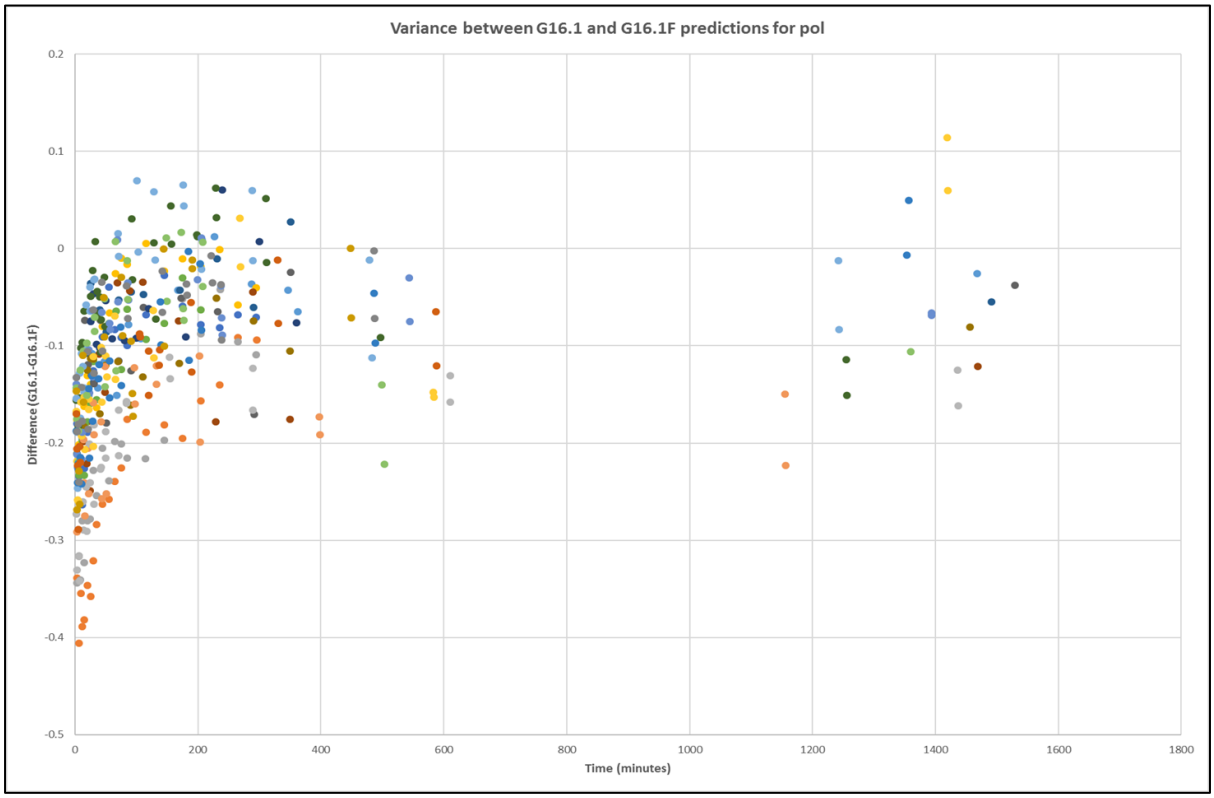

Figure 150: Plot showing the variance between the predicted values for the G16.1 and G16.1 fresh models for pol (each colour represents a different sample)

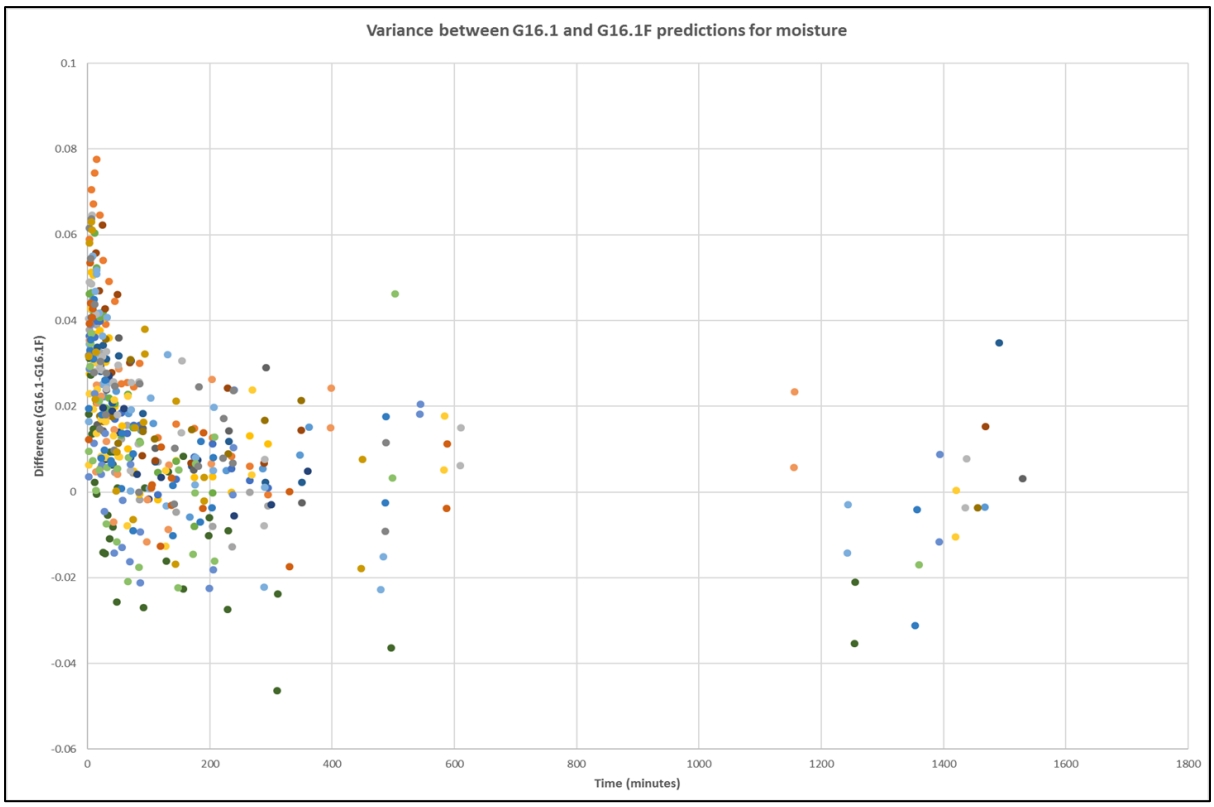

Figure 151: Plot showing the variance between the predicted values for the G16.1 and G16.1 fresh models for moisture (each colour represents a different sample)

The performance of this model in practice provides less scatter and a reduction in skew of the validation set. Figure 152 shows the analysis of shift sugars using the Global 16.1 model and Figure 153 shows the same samples analysed by the G16.1 Fresh models. These samples are of varying age when analysed, from 10 minutes to several hours. The SEP values for the validation set was the same for the two models: 0.11 for the final validation samples.

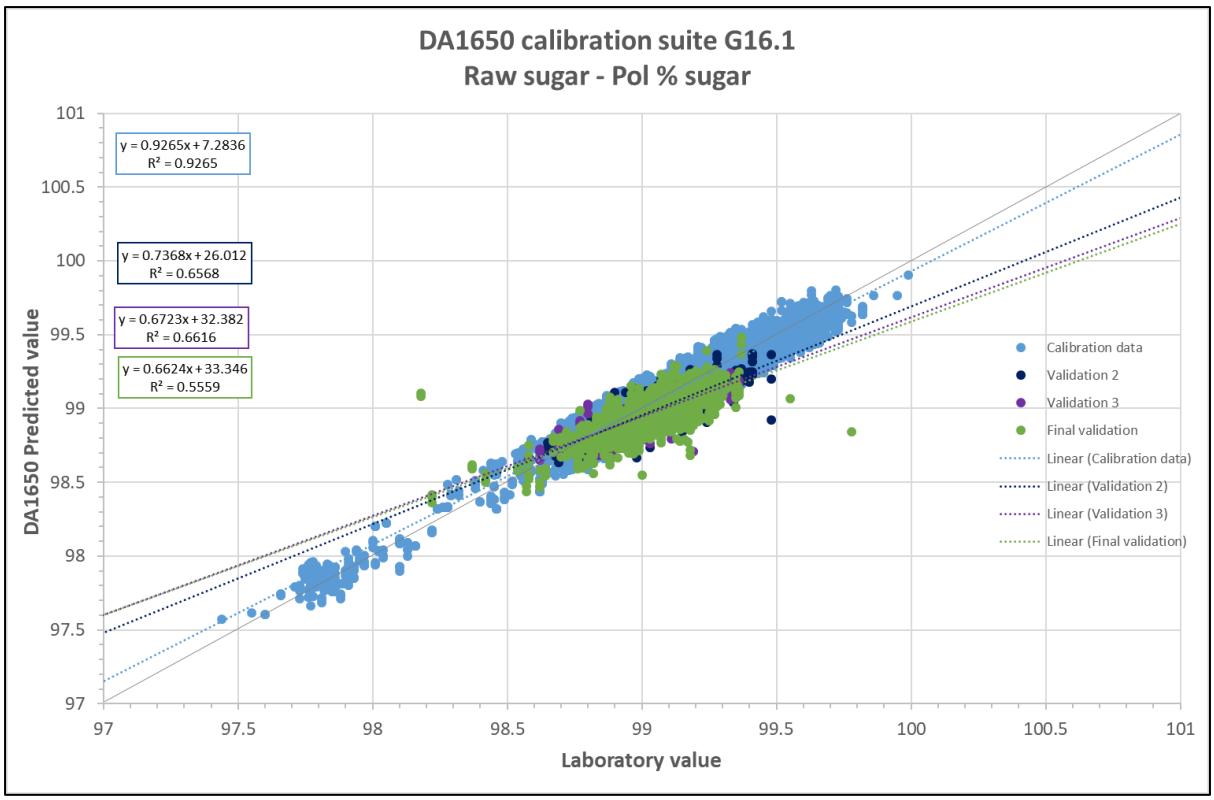

Figure 152: Validation plot for Global 16.1 raw sugar model for pol

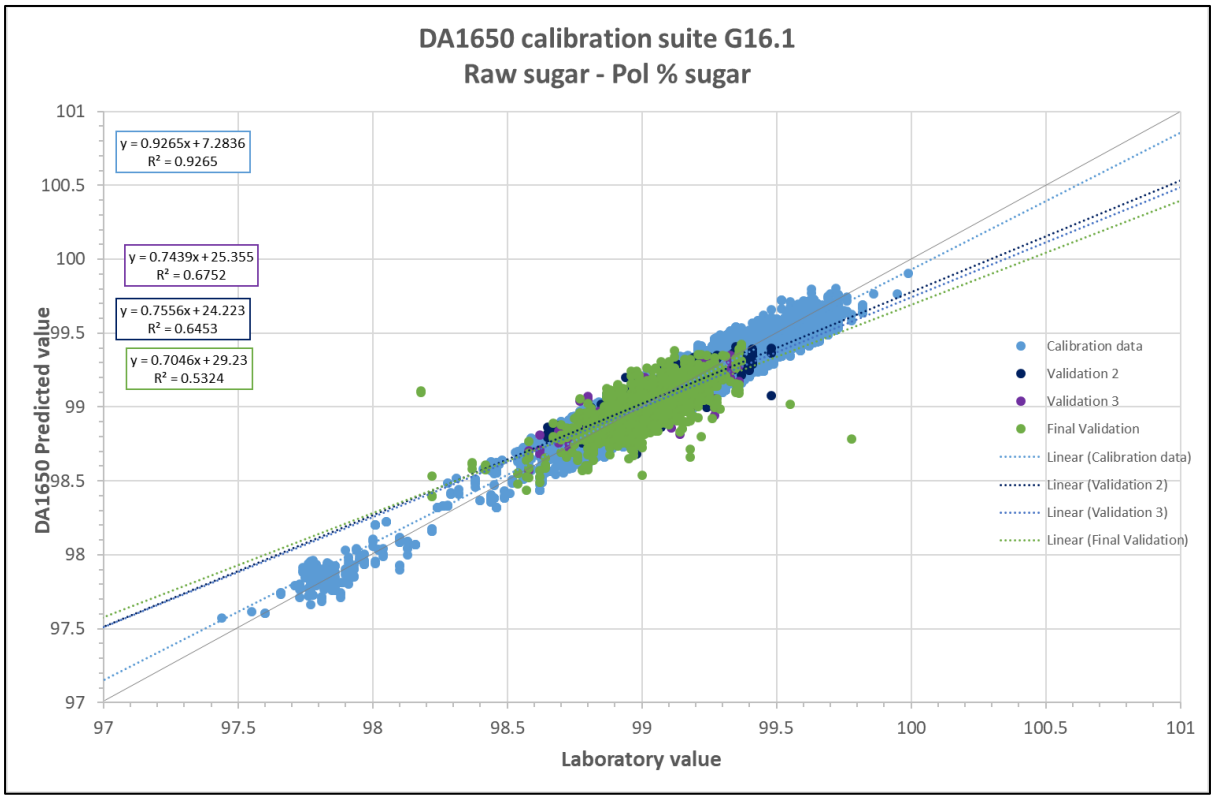

Figure 153: Validation plot for Global 16.1 Fresh raw sugar model for pol

The Global 16.1 Fresh model is still only preliminary, with approximately 20 desensitised sample and 101 regular samples. Despite this, performance is equivalent to the standard equations for fresh samples.

Continued development, focussing on adding mapped samples to capture potential chemical variability in the samples is required and will provide significant improvement to these models.

The success of this technique relied on a very high quality standard prediction model, where the predicted values used as wet chemistry in the calibration set can be trusted.

6.3. Platform performance

This section describes the performance monitoring of the NIR spectroscopic system, including both the hardware and the calibration models. These techniques are used to identify when a calibration needs a bias adjustment or full update and the impact of maintenance on the spectral reproducibility of the instrument.

6.3.1. Standardisation

Despite being factory-manufactured, NIR spectroscopic instruments of the same model and brand vary in ways that affect the spectrum measured. Instrument factors that influence the spectrum include the detector, light source, pathlength, grating, windows and monochromators. This means that calibration models can only be used on the instrument from which their data was originally collected. For applications like those used in the Australian sugar industry, this is impractical as each calibration model can have thousands of spectra and each instrument can run up to 30 calibration models. To re-develop these every time maintenance is performed on an instrument or as the instrument simply ages is not practical.

A significant effort by the NIR community has been invested in improving the reproducibility of the manufacture of instruments and developing mathematical treatments to model the shifts so corrections can be applied (Bouveresse and Massart, 1996). Essentially, this attempts to make the spectra from multiple instruments look the same.

The most successful NIR instrument standardisation method has been Piecewise Direct Standardisation (PDS), which involves measuring a set of 30 sealed samples on each of the instruments. This method has three limitations: (1) sealed samples must represent the material measured in nominal operations; (2) only a very small portion of the measured spectrum is standardised; and (3) the PDS algorithm can only be reliably applied to spectrum absorbencies less than one unit, which is a highly linear region. The grains and pharmaceutical industries use PDS effectively, as sealed samples have a shelf life of several years; the portion of the measured spectrum is highly linear and very few sample types are analysed (Donald, 2013a).

The sugarcane and fresh produce industries are unsuitable for PDS mainly due to the high water content of the samples. High water content poses two problems for NIR measurement: (1) spectral absorbencies are very high, in the region above one unit and which is non-linear; and (2) samples have a shelf life of hours and degenerate rapidly even when refrigerated. Other NIR standardisation methods are inadequate to effectively correct instrument differences for highly absorbing samples such as sugarcane (Donald, 2013a).

At SRA, David Donald and Steve Staunton have been working on developing a suitable standardisation algorithm for online cane analysis systems (CAS) for the last 10 years. To date, no standardisation procedure is available for use in the mills. The background, issues and development of an algorithm has been thoroughly detailed in a suite of internal reports (Donald, 2013a, Donald, 2013b, Donald, 2013c, Donald, 2013d, Donald, 2013e) and will not be repeated here.

David Donald is an international expert on standardisation of NIR instruments. Discussion with David in the early stages of this project identified that a standardisation procedure that is as complex as that being developed for the CAS units was not necessary. Until recently, the instruments installed as CAS units have all been scanning monochromator systems, which are highly variable in build and are influenced strongly by their surrounding environment. The monochromators often required replacement before the life-expectancy. Replacement of a monochromator in an NIR instrument is a major change, and spectrally, was the equivalent of a new instrument.

Recent development in technology has seen the availability of DA-based NIR spectroscopy instruments, such as the DA1650. The benefit of DA instruments is their absence of moving parts, which makes the manufacture more repeatable and there is little to no observable change in response with time. The wavelength response for a DA instrument is particularly stable because each wavelength is read by a pixel on the DA detector.

FOSS has always provided a standardisation mechanism with its instruments, but to date they have not been considered particularly useful. The stability of the DA systems, however, has meant that FOSS' standard standardisation approach is suitable for our mill laboratory applications.

Each FOSS instrument is factory standardised prior to installation at a site. Essentially, all instruments are slaves to a master that is stored in Denmark. Each instrument has an internal standard tile made of spectralon or similar and on every start-up, the tile is scanned and the instrument is subjected to a performance check to ensure the absorbance and wavelength measures are within the expected accuracy and precision. If the performance test fails, or an adjustment is made to the instrument, a standardisation process is initiated. First, an external reference correction is completed using the ERC tool. This corrects for altered intensity and re-calibrates the internal spectralon tile. If the instrument continues to fail its performance checks, an instrument calibration is conducted using the external wavelength correction (EWC) tool, this resets the instrument spectra to the factory conditions.

Typically, a user does not undertake their own adjustments to the instrument standardisation. However, this does not give us the control we need at SRA to provide a suitable service to our clients. Ongoing negotiations with FOSS has resulted in the appropriate tools and standardisation procedures being made available for purchase.

SRA now owns an ERC, and EWC as well as a copy of the official FOSS operational procedures and instructions to conduct instrument standardisation, as required.

As all instruments are factory standardised, all work that has been conducted on the DA1650 instruments has included a standardisation procedure. To date, all calibrations have transferred between instruments (SRA, Tully Mill, QSL) with little to no bias, as has been reported previously. Where a bias has been present, a small selection (n= approx. 30) of samples have been enough to provide a suitable bias adjustment. Bias adjustments appear to be stable over a season.

The value and feasibility of this standardisation procedure has been demonstrated through all of the mill trials. These have demonstrated that the global calibration models are easily and rapidly able to be implemented in new mill settings, with new instrumentation. The standardisation function can be demonstrated with the implementation of the Mill 14 instrument. This system was purchased brand new and required installation into the operating environment for commercial use within a very short turnaround time (days only). Figure 154 shows the performance of the instrument with direct-transfer models. The blue circles are the calibration samples and the orange circles are the preliminary validation samples.

There are only a few points in this group, but they are showing good linearity and only a minor bias from the calibration samples. The green circles represent the orange samples with a bias adjustment applied and the yellow circles represent new samples predicted under the biased calibration. This demonstrates excellent transferability of the models across instruments and supports the standardisation process being used.

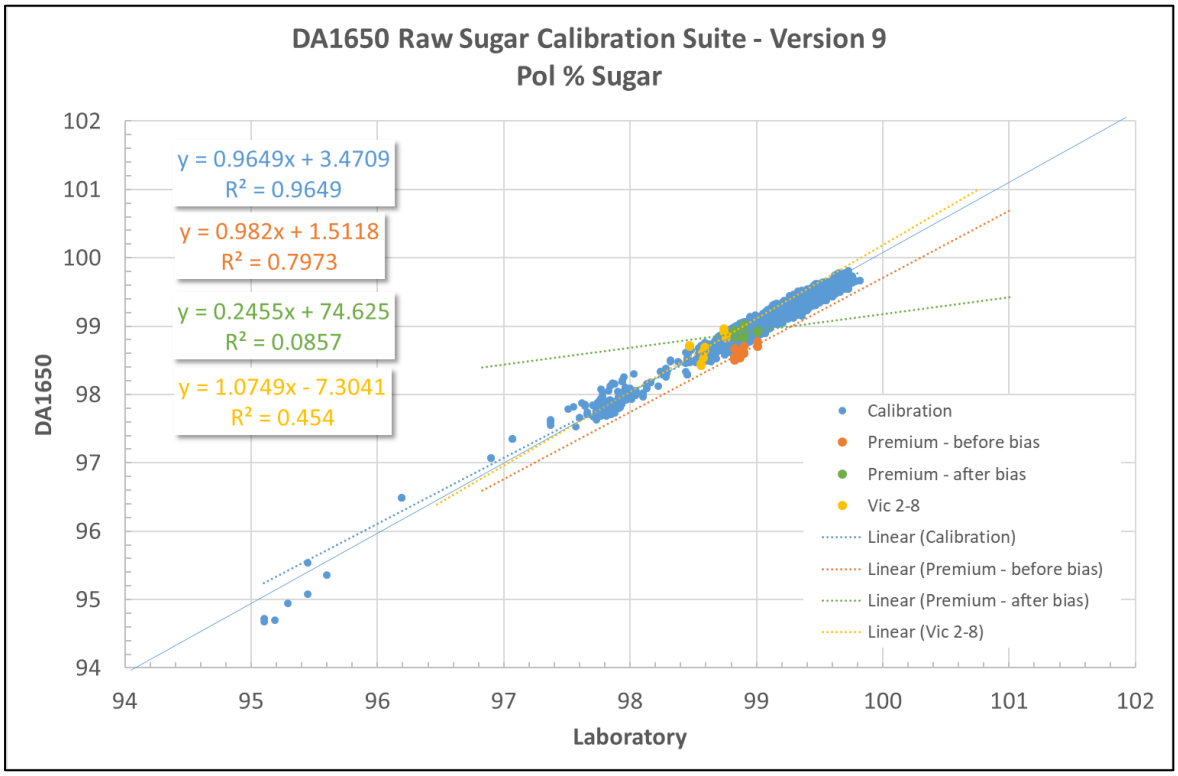

Figure 154: Validation plot for Mill 14 instrument

Additional information on when the EWR and ERC will be applied is provided in Section 6.3.2.4.

6.3.2. Maintenance requirements – hardware

Hardware maintenance describes the actions that ensure the spectra being measured are consistent over time and do not influence the outcome of a predicted result. This is evaluated through analysis of standard reference materials and control charting.

6.3.2.1. Instrument diagnostic test

Every time the instrument is turned on it completes two instrument diagnostic tests: a hardware test and a performance test. The hardware test evaluates the instrument hardware such as reference plate movement, cup motor, lamp voltage and current and instrument communications.
The performance test evaluates the instrument's spectral response against an internal reference tile. Specific tests include evaluation of light path, stability and a wavelength accuracy test.

The diagnostic performance test is pass/fail. The most common reason for failure is inappropriate warm-up time. If the test fails on start-up, the system should be left to warm-up for 15 minutes and the test re-started through the settings menu. If the system fails again, a FOSS Engineer should be contacted. The Diagnostics report provides additional information on the cause of the failure. If it is related to the lamp, a replacement may be required and can be conducted by the user.

The system should not be used if it has failed its instrument diagnostic test.

6.3.2.2. Instrument stability

To date the DA1650 instruments seem to have demonstrated a working robustness and spectral stability, as there have been no significant unexplained shifts in the predicted values. However, all instruments used in the industry over the last three years have been constantly updated with new calibration models, which may be capturing and/or masking any instrument instability.

FOSS provides, with each if its instruments a check cell, which is a sealed cup of rare earth material that provides a standard material against which the instrument performance can be evaluated. The prediction model is specific to each instruments' check cell and returns a measure of distance. Each prediction model has specified bounds within which the instrument is considered to be working effectively (or is stable). When the distance metric is within the warning limit, the distance value shows up yellow on the DA1650 screen. When it is outside the action limit (assuming potential user error such as a dirty read face has been checked), the distance value shows up red on the DA1650 screen and a warning indicates that the system should be checked by a FOSS Engineer.

Over the last three years, check cells have been analysed on both the SRA and Tully Mill instruments, with the distance value returning an acceptable result for each test. A PCA of the untreated spectra of the check cell was conducted to evaluate the variance in the spectra over time. The associated scores plot is provided in Figure 155. This plot shows separation between the Tully Mill and SRA instruments, and distinct grouping of the spectral data by year, particularly for the SRA instrument. It was expected that if there were no change in the instruments with time that for each instrument there would be no grouping of the scores by year, rather they would exist in a cloud that varied randomly. The presence of grouping suggests that there is slight movement of the instrument with time. The impact of this variation on new predictions is difficult to quantify with this data, as PCA will identify any differences in the spectra, however small. This change, as a percentage of sample variation may be insignificant. Causes of this shift may include lamp output, humidity, temperature, electronic noise and/or detector decay. As far as we are aware, this variation has not influenced calibration development and any shift that occurs has been built into the successive calibration updates.

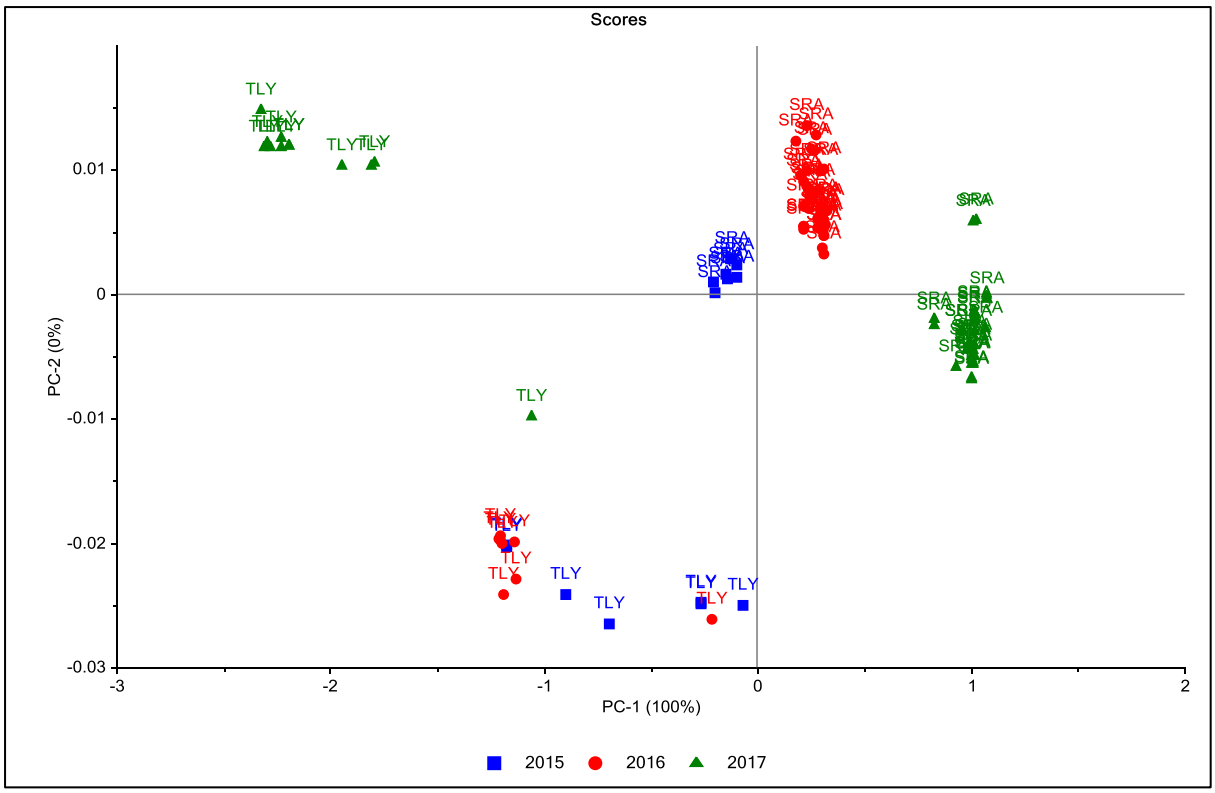

Figure 155: PCA scores plot of untreated check cell spectra for Tully and SRA instruments

To evaluate the magnitude of this shift with respect to a standard calibration model, the spectra were evaluated against the raw sugar pol model. The control chart in Figure 156 demonstrates the variability in instrument performance with time. The dotted warning lines represent two standard deviations of the difference and the solid action line represents three standard deviations of the difference. It is recommended that the check cell values be monitored via a control chart to assist in identifying if the instrument stability is drifting to a point where corrective action is required. The bounds will need to be defined for each system individually and should be calculated on calibration models and systems in standard operating mode (not during calibration development).

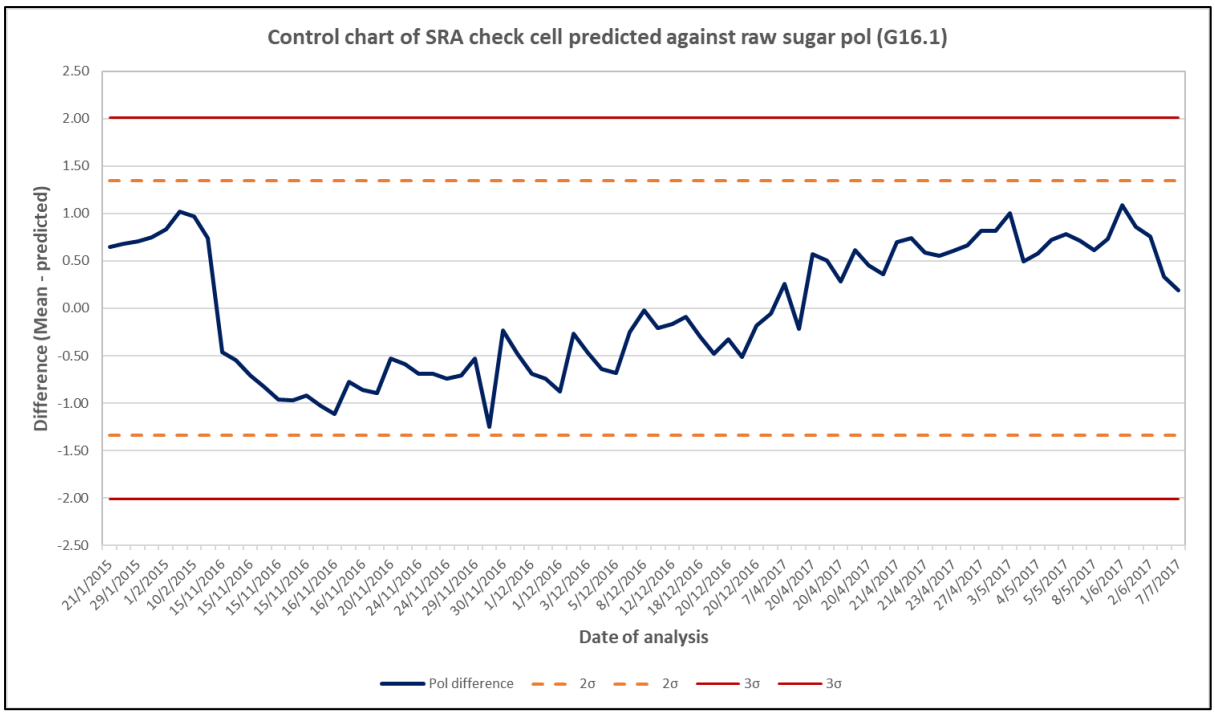

Figure 156: Check sample evaluated against raw sugar pol

The annual shifting of the instrument, along with the results of the mill trials indicate that annual re-calibration is required and should be procedure for the current time.

Additionally, instrument stability can be monitored with the use of sugar-impregnated reference tiles. A set of six fluorilon sugar standards were analysed on the SRA and Tully Mill instruments. First derivative (Gap 5, Segment 3) and SNV spectral treatment removed the instrumental effects and allowed the change in sugar concentration of the standards to be the biggest source of variation in the dataset (Figure 157 and Figure 158).

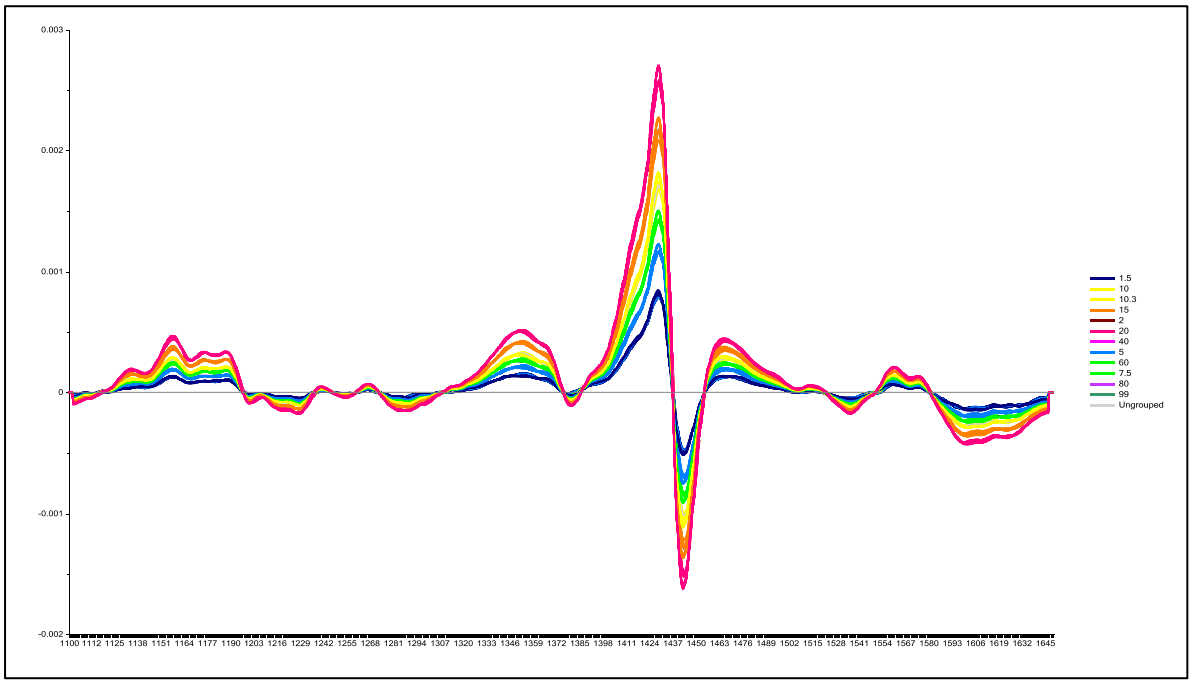

Figure 157: 1SNV spectra of fluorilon reference tiles showing increasing sugar concentration from 1.5 % to 20 %

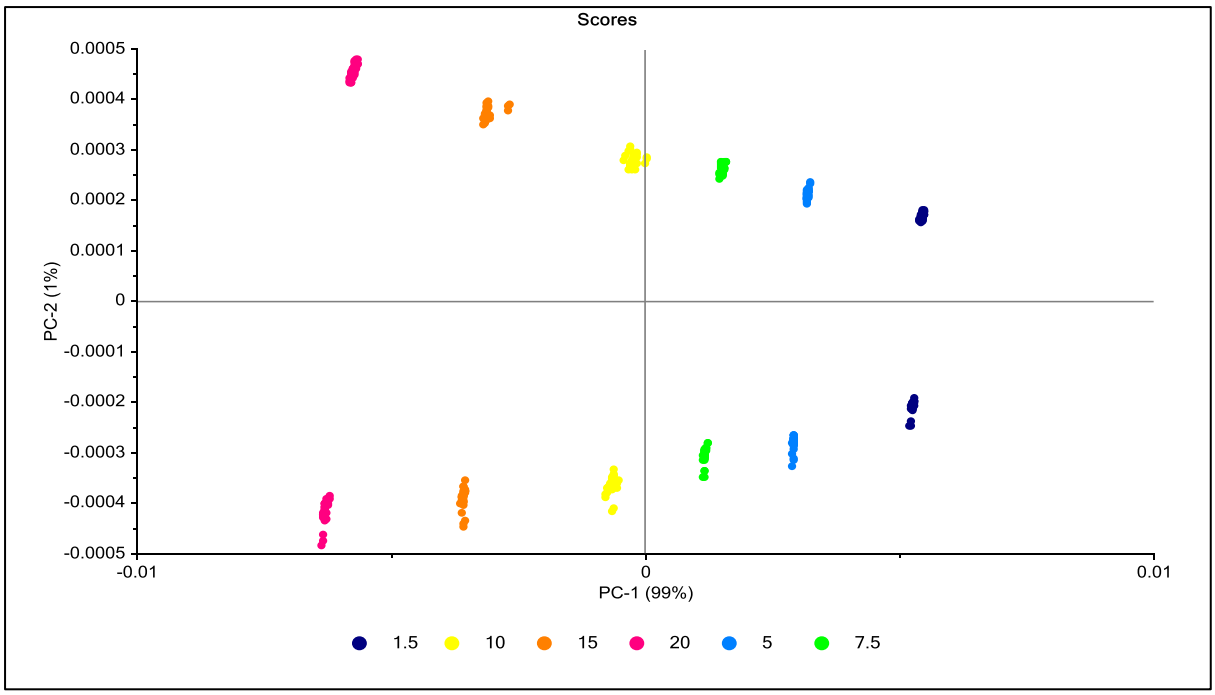

Figure 158: PCA scores plot of 1SNV fluorilon sugar standard spectra

A calibration on the combined Tully Mill and SRA spectra was developed using PLS regression (Figure 159). Validation using an independent set from both instruments gave a very good RMSEP of 0.16, compared to the RMSEC of 0.09. The coefficient of determination for both the calibration and validation was 1.00.

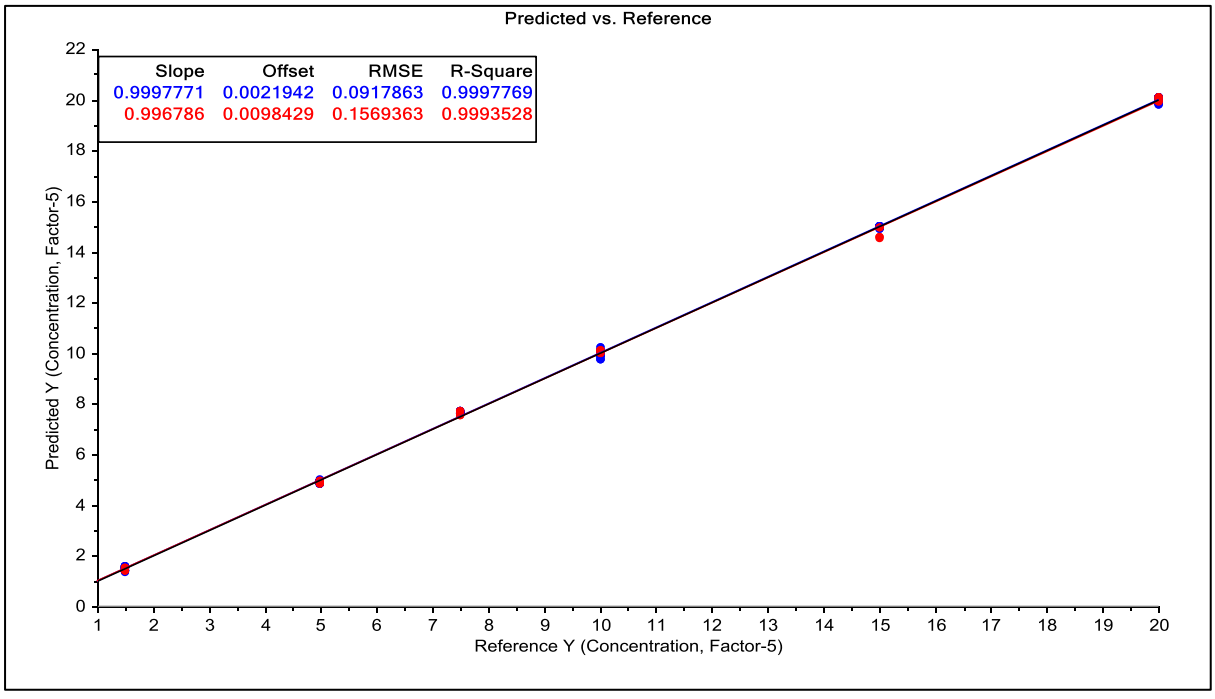

Figure 159: Calibration and independent validation of Fluorilon sugar standards.

These standards are the closest possible material to real sugar factory products that are currently available to provide longitudinal analysis of system performance. It is recommended that these standards be sent to each user in a ring test at least once a year to evaluate system performance.

6.3.2.3. Linearity and photometric tests

As described in Section 1.2, NIR spectroscopy works on the principles of Beer's Law. The linearity of the system is at the most optimal between an absorbance of 0 and 0.7 units, depending on the instrument. Highly absorbing samples, such as those with a large water content, or dark samples such as molasses, can contain spectral regions that sit outside of the linear range of the system. For sugar factory products, most have some or all of their spectrum outside of the linear region of the system (Figure 160). This may increase the error for these samples due to the variability in data collection.

To allow spectral data collection of the wide variety of products that the DA1650 NIR instruments cater for, they are designed to auto-integrate on sample collection. Essentially, this means that the data collection time window for each scan is variable and extends for as long as it takes for enough light to reflect off the sample and be received by the detector. This attempts to automatically optimise the sample collection cased on the absorbance of the sample, minimising some of the non-linearity issues associated with the high absorbance.

Based on previous research at SRA conducted on the ProFOSS™ instruments, fixed scan times would be preferred for all products, however the option is not available on the DA1650 instruments.

Instead, understanding the non-linearity of the instrument is important and we have developed a test protocol to ensure that the instrument is operating appropriately across the operating absorbance range, rather than only at the 99 % reflectance typically evaluated.

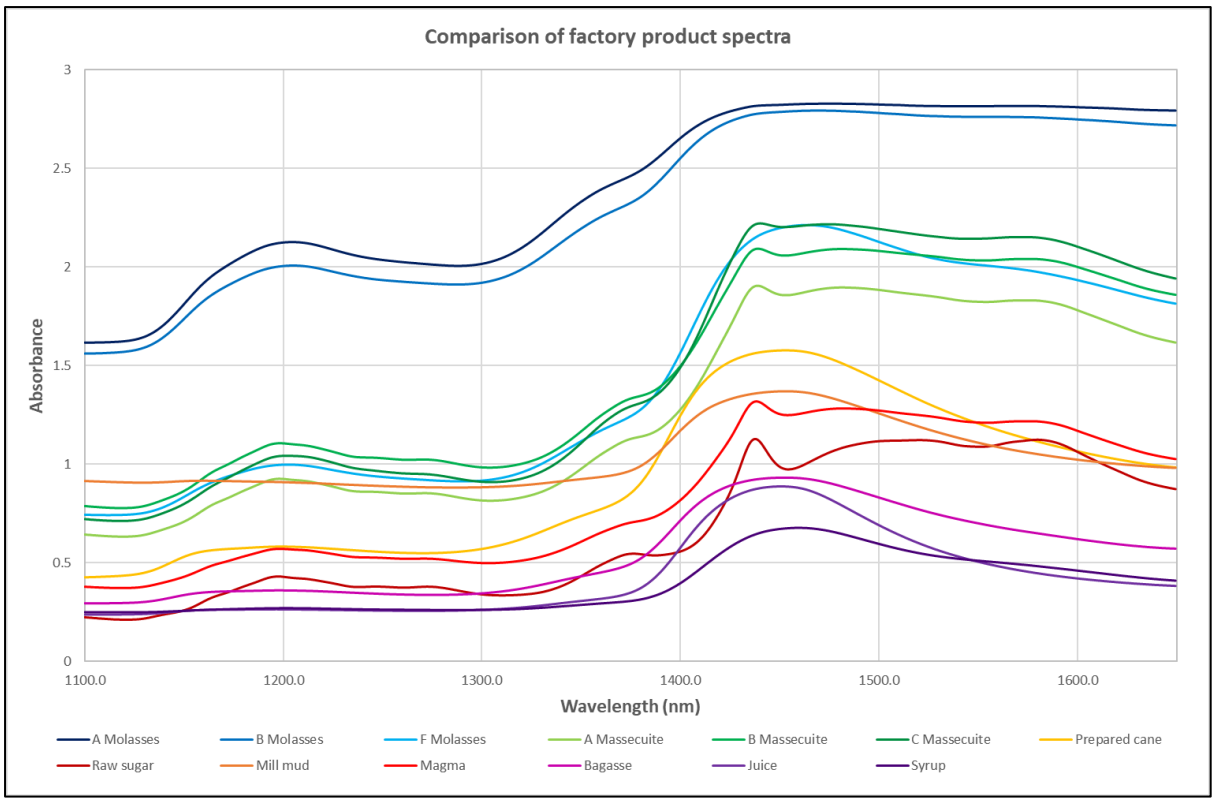

Figure 160: Factory product spectra showing varying absorbance

The test uses a set of photometric standards, which are certified reference tiles of different absorbances (Figure 161). Repeated analysis of these standards on multiple instruments across a period of time provide us with information on the repeatability of the instrument hardware and the performance of the detector across the photometric range. Potentially, this technique can be used to provide non-linear, multipoint standardisation of the instrument network; however, this is outside the scope of this project.

Figure 161: Certified reflectance standards ranging from 2 % to 99 %

The spectra of the reflectance standards cover the absorbance range of most of the factory products, as shown in Figure 162. A and B molasses show absorbances over the 2 % reflectance standard for most of the spectrum. Additionally, almost all of the products have minimum reflectivity of approximately 60 %. This indicates that, if fixed integration were possible the instrument could be tuned for specific products, to force linear-type operation in the 2 % - 60 % reflectance range.

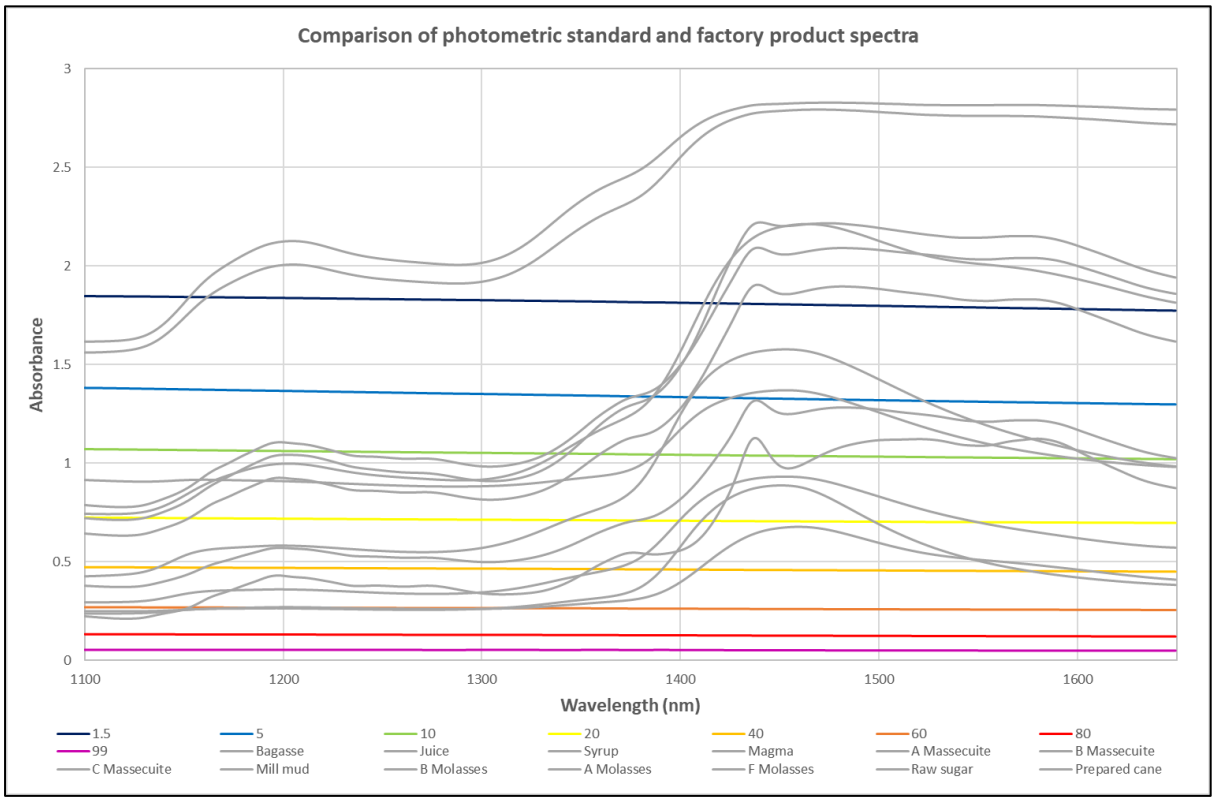

Figure 162: Comparison of factory product spectra and photometric standards

To compare the difference between multiple instruments, the untreated spectra of the photometric standards were subjected to PCA. The associated scores plot is provided in Figure 163. This plot shows that 100 % of the variance in the spectra is due to the change in absorbance of the reference tiles. The SRA and Tully Mill spectra are clustered on top of each other, which indicated that instrumental variability is minimal. The clusters trend from low to high absorbance across PC1, as would be expected. This indicates that a global calibration model may be possible for monitoring ongoing instrument linearity and performance.

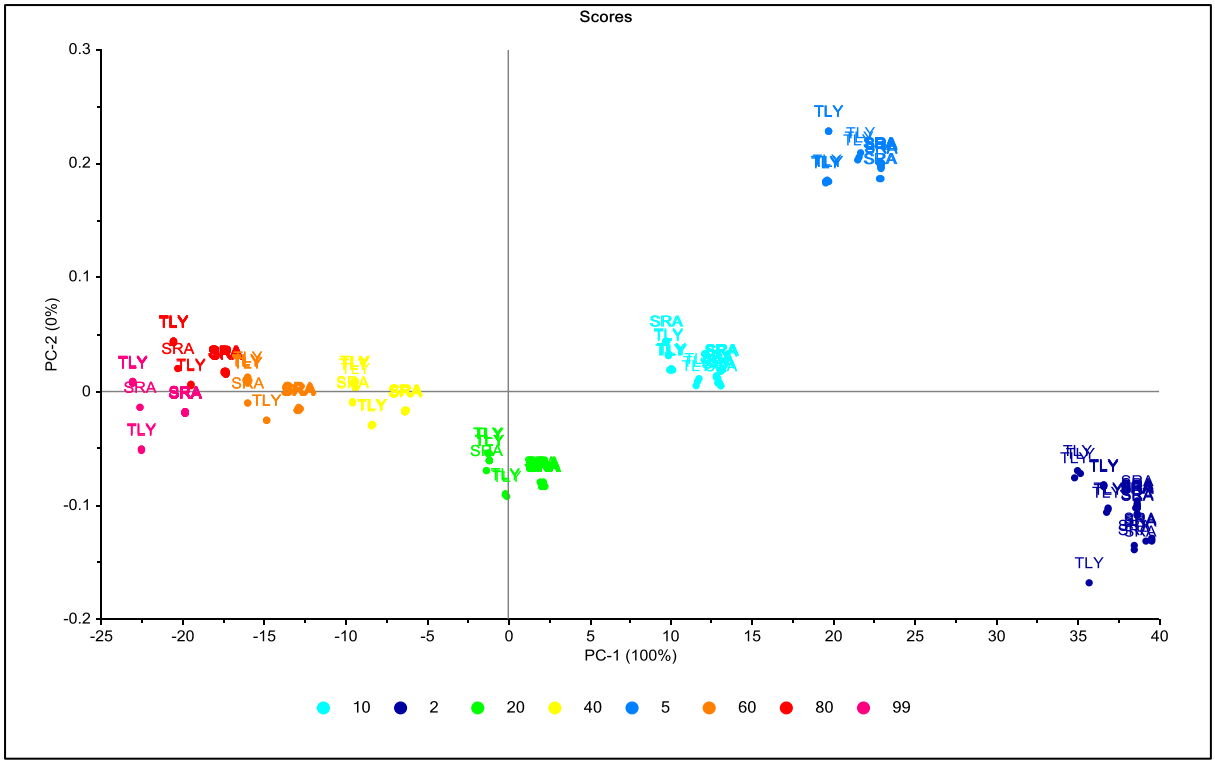

Figure 163: PCA of untreated photometric standard spectra from two instruments

To evaluate the linearity of the system across the photometric range, a calibration curve was developed using PLS. Based on the PCA scores plot, it was expected that one factor would be enough to develop the model. The resulting calibration plot is provided in Figure 164.

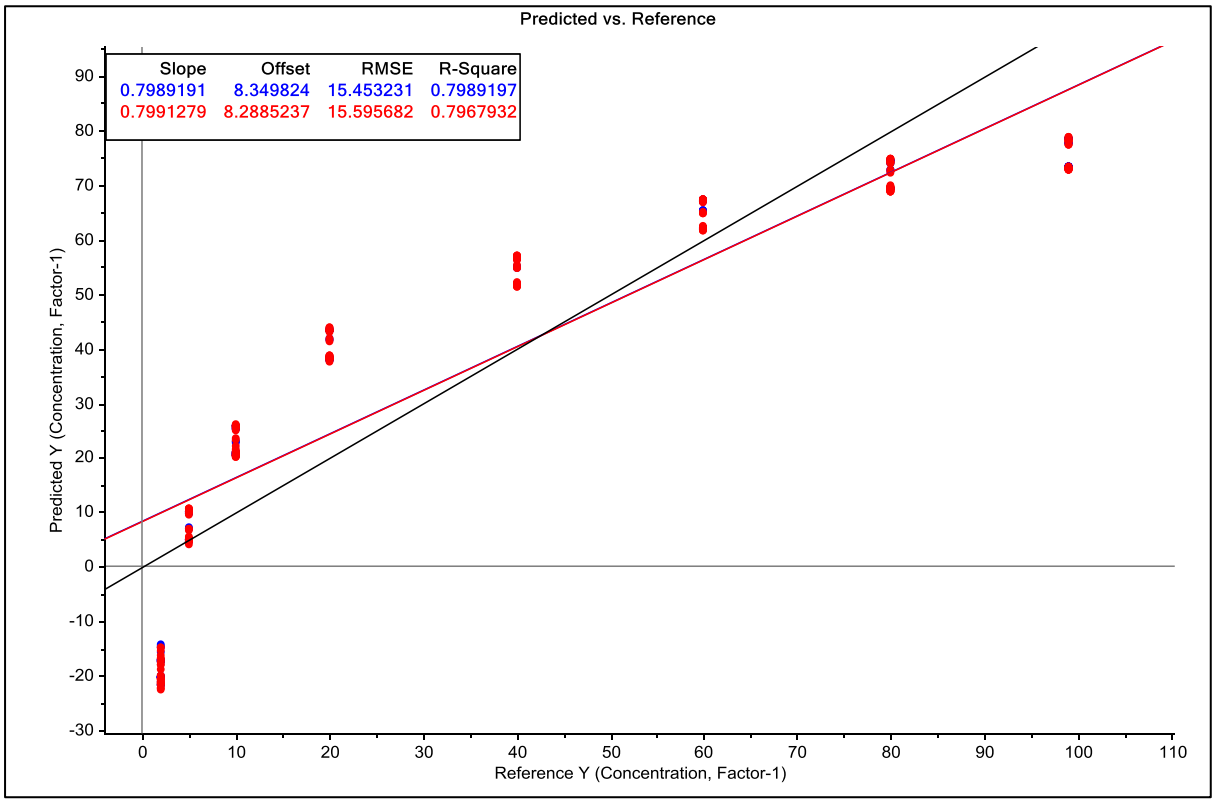

Figure 164: Cross validation calibration/validation plot for photometric standards (1 factor)

This plot indicates that more factors are required to better define the non-linearities of the system. Figure 165 and Figure 166 show the same calibration developed with nine and 16 factors respectively and the show the improved linearity obtained with the addition of model factors. It is possible that the model is being overfit with the inclusion of so many factors; however, the independent validation set is predicted well, suggesting that this is not the case. The 16-factor model is to be used to monitor instrument performance as it contains the same level of model complexity as those used for the analysis of factory products.

The 16-factor calibration curve has an SEC of 2.66 and an SEP value of 2.96. When using this for evaluation of instrument performance. The SEP of the test set should be less than 3.5.

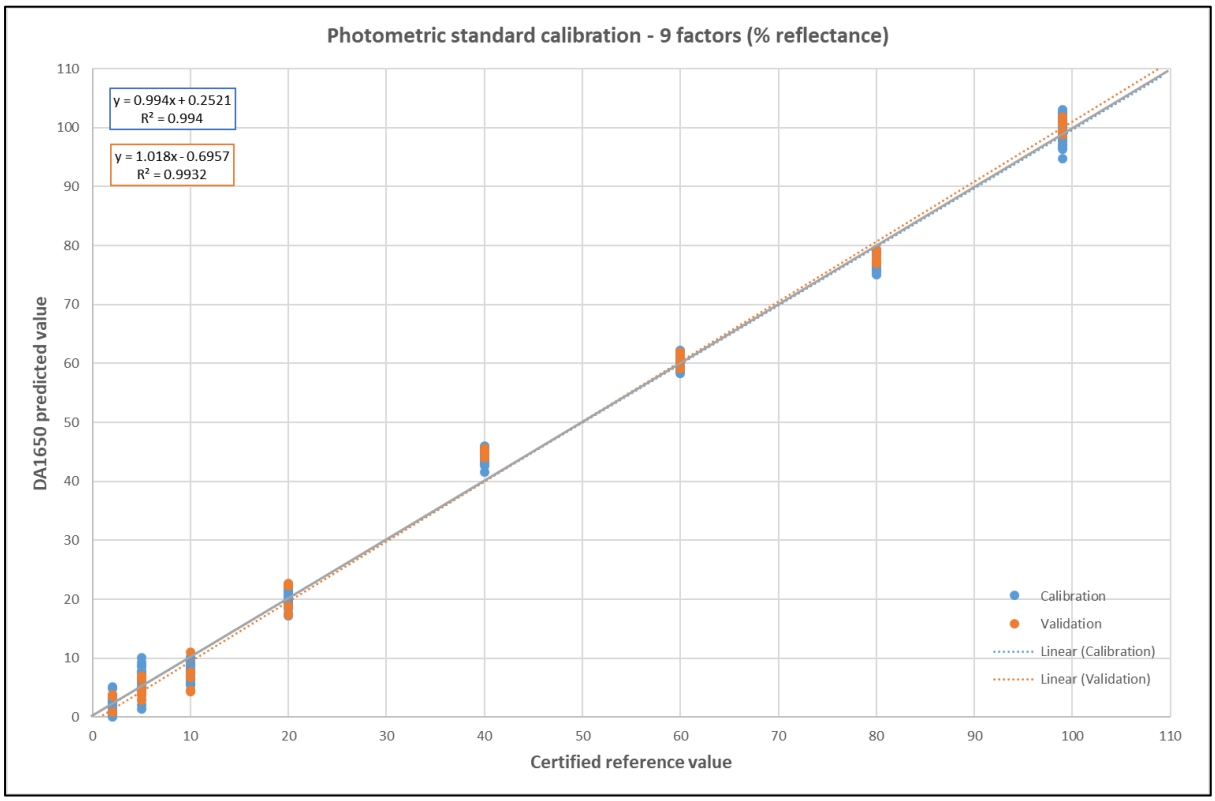

Figure 165: Photometric calibration and independent validation with 9 factors

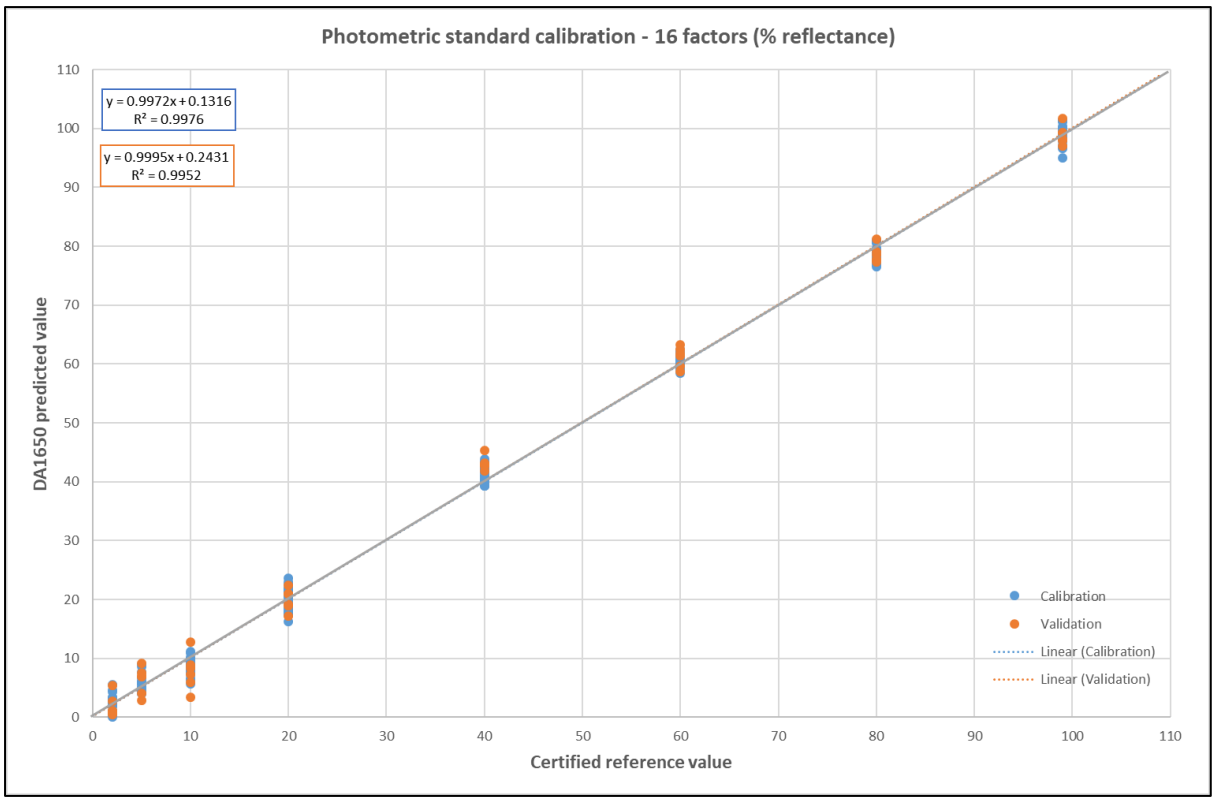

Figure 166: Photometric calibration and independent validation with 16 factors

All instruments should be represented in the global photometric performance test models. Consequently, part of the system installation process should include a period of data collection on the instrument and re-calibration of the model.

6.3.2.4. Instrument calibration (EWC and ERC)

Instrument calibration is the term used by FOSS to describe the standardisation procedure of running the ERC and EWC standards to match the instrument to the Master in Denmark. It ensures consistency of the instrument's photometric, bandpass and wavelength response.

FOSS training material recommends an ERC calibration be conducted following installation, movement to a new site, lamp change, preventative maintenance or repair. If the repair includes replacement of the spectrometer, an EWC calibration should also be conducted. If the diagnostic test fails on wavelength test, an ERC and EWC calibration should be conducted.

However, the product specialists in Denmark recommend only conducting an ERC calibration if the instrument fails its diagnostic tests, which use the internal reference tile. This is because the detector format and the fibre optic-based light management mean that the diode array platforms are more stable than any product calibration models that are run on them and frequent adjusting of the instrument calibration will limit the transferability of the product models.

At SRA, our practice will be to conduct instrument performance tests at a minimum of once per week along with daily check samples. Instrument calibration will be performed if the performance tests fail or major repairs have been made to the internals of the instrument, which may have affected the analysis conditions of the instrument.

6.3.2.5. Known hardware issues

Throughout the project, several minor hardware issues occurred and were solved with assistance from FOSS' Engineering Support. They included:

- Mismatched time stamp – If the time on the instrument drifts more than five minutes, it will not connect to Mosaic and sync properly. This can be fixed by correcting the time in the *Instrument Information* section of the care menu
- Failure of touch screen – During this project, a factory replacement of the touch screens was conducted due to frequent failure. The replacement screens have a better seal around the edge to prevent water and factory product ingress. Care should be taken to regularly clean the screen with a damp (not wet) cloth and use of a brush to remove sugar crystals from the base of the screen. If the touch screen fails, a mouse can be connected for use until a FOSS Engineer can be contacted.
- Full memory – The instruments only have a small amount of storage capacity. Periodically, the memory must be cleared for additional products to be analysed. This can be done by the User or remotely by a FOSS Engineer.

6.3.3. Maintenance requirements – calibration models

Calibration models should be monitored through validation sampling. A minimum of 10 % of the samples subjected to NIR spectroscopic analysis should also be analysed through the traditional wet chemistry techniques and the results of the two techniques compared. Consistent or increasing deviation between the NIR predicted values and the reference values suggests that the calibration is no longer appropriately predicting new samples. Validation monitoring is achieved through linear regression, control charting and evaluation of validation statistics. Each of these metrics are described in the affiliated industry report for benchtop NIR spectroscopic systems (Keeffe and Staunton, 2017).

If the validation residuals begin to move out of control after a period of stability, the calibration model should be updated. Before this is done, an evaluation should be made as to whether the variation could be due to changes in reference analyses, unintended changes in measuring conditions (e.g. caused by process changes), instrument drift or malfunction, operation and maintenance of laboratory equipment, or similar. In some cases, a simple adjustment of the constant term in the calibration equation may be sufficient (bias adjustment). In other cases, it may be necessary to run a re-calibration procedure, where the calibration set is expanded to include samples from the running validation.

Improved end-user reporting to be developed following the conclusion of this project will allow control charts to be automatically developed to display the residuals between the wet chemistry and reference values, or, check samples and their 'ideal' spectrum. Once this is available, calibrations will be updated based on standard control charting statistics.

7. PUBLICATIONS

To date, no formal publications have been finalised from this project, however, an ASSCT paper will be prepared for the 2018 conference and the Industry User Manual, *Overview of benchtop near infrared (NIR) spectroscopic applications in the sugarcane mill environment* is currently under review.

8. REFERENCES

2000. Understanding spectra, Improving processes. *In:* BRUKER (ed.).

2001a. The laboratory manual for Australian sugar mills. Brisbane, Australia: BSES Publications.

2001b. The laboratory manual for Australian sugar mills. Brisbane, Australia: BSES Publications.

2015. The Unscrambler® X version 10.4 - Help documentation. Norway: CAMO Software AS.

AMES, R. V., NORTON, S. W. & NGUYEN, H. H. 1989a. Control of sugar polarisation and moisture using near infared reflectance analysis. *Proceedings of the Australian Society of Sugar Cane Technologists***,** 246-252.

AMES, R. V., NORTON, S. W. & NGUYEN, H. H. Control of sugar polarisation and moisture using near infrared reflectance analysis. Proceedings of the Australian society of sugar cane technologists, 1989b. 246-252.

BARTENS, A. 2011. Methods Book. Germany: International Commission for Uniform Methods of Sugar Analysis.

BERDING, N. Proceedings of the 7th International conference of NIR spectroscopy, 1995 Montreal.

BERDING, N. & BROTHERTON, G. A. 1994. Leaping ahead with NIR spectroscopy. *Proceedings of the 6th international conference of NIR spectroscopy.*

BERDING, N., BROTHERTON, G. A., LE BROCQ, D. G. & SKINNER, J. C. 1991. Near infrared reflectance spectroscopy for analysis of sugarcane from clonal evaluation trials: I. Fibrated cane. *Crop Science,* 31**,** 1017-1023.

BEVIN, C., STAUNTON, S. P., STOBIE, R., KINGSTON, J. & LONERGAN, G. 2002. On-line use of near infrared spectroscopy in a sugar analysis system (SAS). *Proceedings of the Australian Society of Sugar Cane Technologists,* 24.

BILBA, K. & OUENSANGA, A. 1996. Fourier transform infrared spectroscopic study of thermal degradation of sugar cane bagasse. *Journal of Analytical and Applied Pyrolysis,* 38**,** 61-73.

BOUVERESSE, E. & MASSART, D. L. 1996. Standardisation of near-infrared spectrometric instruments: A review. *Vibrational Spectroscopy,* 11**,** 3-15.

BRIZUELA, A. B., BICHARA, L. C., ROMANO, E., YURQUINA, A., LOCATELLI, S. & BRANDÁN, S. A. 2012. A complete characterization of the vibrational spectra of sucrose. *Carbohydrate Research,* 361**,** 212-218.

BROTHERTON, G. A. & BERDING, N. 1995. Near infra-red spectroscopic applications for milling: prospects and implications. *Proceedings of the Australian Society of Sugar Cane Technologists,* 17**,** 21-29.

BROTHERTON, G. A. & BERDING, N. 1998. At-line analysis of mill-prepared cane using near infra-red spectroscopy. *Proceedings of the Australian Society of Sugar Cane Technologists,* 20**,** 34-42.

CAO, N. 2013. *Calibration optimization strategy and efficiency in near infrared spectrpscopy.* Doctor of Philosophy, Iowa State University.

CHANG, C.-W., LAIRD, D. A., MAUSBACH, M. J. & HURBURGH, C. R. 2001. Near-Infrared Reflectance Spectroscopy–Principal Components Regression Analyses of Soil Properties Journal Paper no. J-18766 of the Iowa Agric. and Home Econ. Exp. Stn., Ames, IA. *Soil Sci. Soc. Am. J.,* 65**,** 480-490.

CHAPLIN, M. 2000. *Water absorption spectrum* [Online]. Available: http://www1.lsbu.ac.uk/water/water_vibrational_spectrum.html [Accessed].

CHEN, J. C. P. & CHOU, C. C. 1993. *Cane Sugar Handbook: A Manual for Cane Sugar Manufacturers and Their Chemists*, Wiley.

CHEN, M., GLAZ, B., GILBERT, R. A., DAROUB, S. H., BARTON II, F. E. & WAN, Y. 2002. Near infrared reflectance spectroscopy analysis of phosphorus in sugarcane leaves. *Agronomy Journal,* 94**,** 1324-1331.

CHONG, B. F. & O'SHEA, M. G. 2012. Developing sugarcane lignocellulosic biorefineries: opportunities and challenges. *Biofuels,* 3**,** 307-319.

CLARK, D. H., MAYLAND, H. F. & LAMB, R. C. 1987. Mineral analysis of forages with near infrared reflectance spectroscopy. *Agronomy Journal,* 79 485-490.

CLARKE, M., EDYE, L. & PATOUT, W. Proceedings of the International Society of Sugar Cane Technologists, 1995. 44.

CLARKE, M., LEGENDRE, B., PERDOMO, R., EDYE, L., BLANCO, R., MIRANDA, X. & KELLY, A. Proc. SPRI, 1994 Finland. 358.

CLARKE, M. A., ARIAS, E. & MCDONALD-LEWIS, C. NIR analysis in the sugarcane factory. Proceedings of the International Society of Sugar Cane Technologists, 1992. 829-841.

COUILLARD, A. 1996. Determination of soil seperates with near infrared reflectance spectroscopy. *Journal of Near Infrared Spectroscopy,* 4**,** 201-212.

COZZOLINO, D. & MORON, A. 2006. Potential of near-infrared reflectance spectroscopy and chemometrics to predict soil organic carbon fractions. *Soil and Tillage Research,* 85**,** 78-85.

DAVIES, A. M. C. & MILLER, C. E. 1988. Tentative Assignment of the 1440-nm Absorption Band in the Near-Infrared Spectrum of Crystalline Sucrose. *Applied Spectroscopy,* 42**,** 703-704.

DAY-LEWIS, C. 1994. The use of near infrared (NIR) in sugar factories. Durban, South Africa: Sugar Milling Research Institute.

DONALD, D. 2013a. Standardisation of Sugar-Industry Near-Infrared Instrumentation: Part 1 Standardisation Algorithm. Gordonvale, QLD: Sugar Research Australia.

DONALD, D. 2013b. Standardisation of Sugar-Industry Near-Infrared Instrumentation: Part 2 Photometric Standards, Measurements and Linearisation. Gordonvale, QLD: Sugar Research Australia.

DONALD, D. 2013c. Standardisation of Sugar-Industry Near-Infrared Instrumentation: Part 3 Multiple Photometric Standards. Gordonvale, QLD: Sugar Research Australia.

DONALD, D. 2013d. Standardisation of Sugar-Industry Near-Infrared Instrumentation: Part 4 Standardised CAS Network. Gordonvale, QLD: Sugar Research Australia.

DONALD, D. 2013e. Standardisation of Sugar-Industry Near-Infrared Instrumentation: Part 5 A False Chute. Gordonvale, QLD: Sugar Research Australia.

DONALD, D., GRAHAM, B. G., STAUNTON, S. P., SIMPSON, J. & O'SHEA, M. G. 2013. Routine data generation for sugar mill and refinery applications using a laboratory-based near infrared (NIR) instrument. *Proceedings of the Australian Society of Sugar Cane Technologists.* www.assct.com.au: Australian Society of Sugar Cane Technologists.

DU, C. & ZHOU, J. 2009. Evaluation of soil fertility using infrared spectroscopy: a review. *Environmental Chemistry Letters,* 7**,** 97-113.

FONG CHONG, B., PURCELL, D. & O'SHEA, M. 2013. Diffuse Reflectance, Near-Infrared Spectroscopic Estimation of Sugarcane Lignocellulose Components—Effect of Sample Preparation and Calibration Approach. *BioEnergy Research,* 6**,** 153-165.

GELADI, P., MACDOUGALL, D. & MARTENS, H. 1985. Linearization and Scatter-Correction for Near-Infrared Reflectance Spectra of Meat. *Appl. Spectrosc.,* 39**,** 491-500.

GUILHERME, A. A., DANTAS, P. V. F., SANTOS, E. S., FERNANDES, F. A. N. & MACEDO, G. R. 2015. EVALUATION OF COMPOSITION, CHARACTERIZATION AND ENZYMATIC HYDROLYSIS OF PRETREATED SUGAR CANE BAGASSE. *Brazilian Journal of Chemical Engineering,* 32**,** 23-33.

HALGERSON, J. L., SHEAFFER, C. C., MARTIN, N. P., PETERSON, P. R. & WESTON, S. J. 2004. Near-Infrared Reflectance Spectroscopy Prediction of Leaf and Mineral Concentrations in Alfalfa *Agronomy Journal,* 96**,** 344-351.

HARRIS, D. C. 2007. *Quantitative Chemical Analysis,* New York, USA, W. H. Freeman and COmpany.

HART, J. R., NORRIS, K. H. & GOLUMBIC, C. 1962. Determination of the moisture content of seeds by near-infrared spectroscopy of their methanol extracts. *Cereal Chemistry,* 39**,** 94-99.

HIRSCHFIELD, T. Salinity determination using NIRA. *Applied spectroscopy,* 39**,** 740-741.

HUANG, G., HANA, L. & LIUA, X. 2007. Rapid estimation of the composition of animal manure compost by near infrared reflectance spectroscopy. *Journal of Near Infrared Spectroscopy,* 15**,** 387-394.

HUANG, H., YU, H., XU, H. & YING, Y. 2008. Near infrared spectroscopy for on/in-line monitoring of quality in foods and beverages: A review. *Journal of Food Engineering,* 87**,** 303-313.

HUBER, L. 2010. Validation of Analytical Methods. Germany: Agilent Technologies.

IWAMOTO, M., UOZUMI, J. & NISHINARI, K. 1986. Preliminary investigation of the state of water in foods by near infrared spectroscopy. *International NIR/NIT Conference.* Budapest, Hungary.

JOHNSON, T. P. 2001. Cane juice analysis by near infrared (NIR) to determine grower payment. *Journal American Society of SUgar Cane Technologists,* 21**,** 62-72.

KANNAR, D., KITCHEN, B. J. & O'SHEA, M. 2010. Process for the Manufacture of Sugar and Other Food Products. Google Patents.

KEEFFE, E. C. 2013. *Rapid nutrient determination of sugarcane milling by-products using near infrared spectroscopy.* Master of Applied Science (Research), Queensland University of Technology.

KEEFFE, E. C., OSTATEK- BOCZYNSKI, Z. A., DONNAN, L. J. & O'SHEA, M. Implementing NIR spectroscopic systems for determining the nutrient content of mill by-products: Validation trials (Poster). Australian Society of Sugar Cane Technologists, 2014.

KEEFFE, E. C., OSTATEK- BOCZYNSKI, Z. A., DONNAN, L. J. & O'SHEA, M. 2015. Using rapid analytical methods to quantify nutrients in sugarcane leaf tissue. *International Sugar Journal,* (in press).

KEEFFE, E. C., OSTATEK-BOCZYNSKI, Z. A., DONNAN, L. J., MARTENS, W. N. & O'SHEA, M. G. 2013. Developing, validating and applying near infrared (NIR) spectroscopic methods for the nutrient compositional analysis of sugar mill byproducts. *Proceedings of the Australian Society of Sugar Cane Technologists.* Australian Society of Sugar Cane Technologists.

KEEFFE, E. C. & STAUNTON, S. 2017. Overview of benchtop near infrared (NIR) spectroscopic applications in the sugarcane mill environment. Brisbane, Australia: Sugar Research Australia.

LARRAHONDO, J. E., PALAU, F., NAVARRETE, A. & RAMÍREZ, C. Applications of near infrared spectroscopy in the sugarcane industry of Colombia. *In:* HOGARTH, D. M., ed. International Society of Sugar Cane Technologists: XXIV Congress, 2001 Brisbane, Australia. 163-65.

LIN, J. & BROWN, C. W. 1994. Novel applications of near-infrared spectroscopy of water and aqueous solutions from physical chemistry to analytical chemistry. *TrAC Trends in Analytical Chemistry,* 13**,** 320-326.

LLOYD, T., EASTMENT, S. & MITCHELL, P. Milling train maceration control utilising NIR technology. Proceeding of the Australian Society of SUgar Cane Technologists, 2010. 688-695.

MADSEN II, L. R., WHITE, B. E. & REIN, P. W. 2003. Evaluation of a near infrared spectrometer for the direct analysis of sugar cane. *Journal of the American society of sugarcane technologists,* 23**,** 80-92.

MALLEY, D. F. & VANDENBYLLAARDT, L. 1999. Feasibility Study on the Rapid Compositional Analysis of Hog Manure by Near-infrared Spectroscopy.

MARTENS, H. & NÆS, T. 1992. *Multivariate Calibration*, Wiley.

MASSART, D. L. 1998. *Handbook of chemometrics and qualimetrics*, Elsevier.

MASSIE, D. R. & NORRIS, K. H. 1964. The spectral reflectance and transmittance properties of grain in the visible and near-infrared. *Trans. Am. Soc. Agric. Eng.,* 84**,** 598-600.

MEYER, J. H. 1997. Review of near infra-red spectroscopy research in the South African sugar industry. *Proceedings of the South African Sugar Technologists Association* 71**,** 33-37.

MEYER, J. H. & KEEPING, M. G. 2001. Chapter 16 Past, present and future research of the role of silicon for sugarcane in southern Africa. *In:* L.E. DATNOFF, G. H. S. & KORNDÖRFER, G. H. (eds.) *Studies in Plant Science.* Elsevier.

MEYER, J. H. & WOOD, R. A. Rapid analysis of cane juice by NIR. Proc. South Afr. Sugar Technol. Assoc., 1988. 203-207.

MILLER, C. E. 2001. Chemical principles of near-infrared technology. *In:* WILLIAMS, P. & NORRIS, K. H. (eds.) *Near-infrared technology in the agricultural and food industries.* 2 ed. St Paul, Minnesota: American Association of Cereal Chemists.

MLOTKIEWICZ, J. A. 2005. The Role of the Maillard Reaction in the Food Industry. *In:* NURSTEN, H. E., CRABBE, M. J. C. & AMES, J. M. (eds.) *The Maillard Reaction in Foods and Medicine.* Woodhead Publishing.

MOLT, K., BERENTSEN, S., FROST, V. J. & NIEMOELLER, A. 1998. NIR spectrometry- An alternative for the analysis of aqueous systems. *Spectroscopy Europe,* 10**,** 16-21.

MORTARI, D. A., ÁVILA, I., SANTOS, A. M. & CRNKOVIC, P. C. G. M. 2012. Study of thermal decomposition of ignition temperature of bagasse, coal and their blends. *Engenharia Térmica,* 91-281-88.

MUNIR, S., DAOOD, S. S., NIMMO, W., CUNLIFFE, A. M. & GIBBS, B. M. 2009. Thermal analysis and devolatilization kinetics of cotton stalk, sugar cane bagasse and shea meal under nitrogen and air atmospheres. *Bioresource Technology,* 100**,** 1413-1418.

MURRAY, I. & WILLIAMS, P. C. 1987. Chemical principles of near-infrared technology. *In:* WILLIAMS, P. & NORRIS, K. (eds.) *Near-infrared technology in the agricultural and food indusries.* St. Paul, Minnesota, USA: American Association of Cereal Chemists.

NAWI, N., CHEN, G. & JENSEN, T. 2014. In-field measurement and sampling technologies for monitoring quality in the sugarcane industry: a review. *Precision agriculture,* 15**,** 684-703.

NAWI, N. M., CHEN, G. & JENSEN, J. P. Visible and shortwave near infrared spectroscopiy to predict sugarcane quality from clear and raw juice samples. *In:* NOOR, M. M., RAHMAN, M. M. & ISMAIL, J., eds. 3rd Malaysian Postgraduate Conference, 2013 Sydney NSW, Australia. 167-174.

NAWI, N. M., JENSEN, T. & CHEN, G. 2012. The application of spectroscopic methods to predict sugrcane quality based on stalk cross-sectional scanning. *Journal American Society of Sugar Cane Technologists,* 32**,** 16-27.

NIU, Q., ZONG, Y., QIAN, M., YANG, F. & TENG, Y. 2014. Simultaneous quantitative determination of major plant hormones in pear flowers and fruit by UPLC/ESI-MS/MS. *Analytical Methods,* 6**,** 1766-1773.

NOLASCO, J. & DE MASSAGUER, P. R. 2006. THERMAL DEGRADATION KINETICS OF SUCROSE, GLUCOSE AND FRUCTOSE IN SUGARCANE MUST FOR BIOETHANOL PRODUCTION. *Journal of Food Process Engineering,* 29**,** 462-477.

NORRIS, K. H., BARNES, R. F., MOORE, J. E. & SHENK, J. S. 1976. Predicting Forage Quality by Infrared Reflectance Spectroscopy. *Journal of Animal Science,* 43**,** 889-897.

NORRIS, K. H. & HART, J. R. Direct spectrophotometric determination fo moisture content of grain and seeds. Proc. 1963 :Int. Symp. Humidity Moisture, 1965 New York. Reinhold, 19-25.

O'SHEA, M., KEEFFE, E., BURNS, E. & STAUNTON, S. An analysis of the lignocellulosic diversity of sugarcane. Proceedings of the XXVII ISSCT Congress, Sao Paulo, Brazil, 24-27 June, 2013., 2013a. (USB), 28: 11p.

O'SHEA, M., OSTATEK-BOCZYNSKI, Z., KEEFFE, E. & MARTENS, W. 2013b. Development of near infrared (NIR) spectroscopic methods to predict carbon, nitrogen, silicon, phosphorus and potassium levels in mill by-products. *International Sugar Journal,* 115**,** 656-660.

O'SHEA, M. G., STAUNTON, S. P. & BURLEIGH, M. 2010. Implementation of on-line near infrared (NIR) technologies for the analysis of cane, bagasse and raw sugar in sugar factories to improve performance. *Proceedings of the international society of sugar cane technologists,* 27**,** (CD-ROM) 13pp.

O'SHEA, M. G., STAUNTON, S. P., DONALD, D. & SIMPSON, J. Developing laboratory near infra-red (NIR) instruments for the analysis of sugarcane factory products. Proceedings of the Australian Society of Sugar Cane Technologists, 2011.

O'SHEA, M. G., DONALD, D., SIMPSON, J., KEEFFE, E. C. & STAUNTON, S. 2014. Development of laboratory near infrared (NIR) spectroscopy systems for sugar mill and refinery applications. *International Sugar Journal***,** 748-753.

OXLEY, J., FONG CHONG, B., SANT, G. G. & O'SHEA, M. G. Accelerating the characterisation of sugarcane biomass using near-infrared (NIR) spectroscopic techniques. Proceedings for the Australian Society of Sugar Cane Technologists, 2012 Cairns, Australia.

PAX, R. A. NIR: Its use and abuse- Case study of B molasses. Proceedings of the australian society of sugar cane technologists, 1993. 202-207.

PISSARD, A., FERNÁNDEZ PIERNA, J. A., BAETEN, V., SINNAEVE, G., LOGNAY, G., MOUTEAU, A., DUPONT, P., RONDIA, A. & LATEUR, M. 2013. Non-destructive measurement of vitamin C, total polyphenol and sugar content in apples using near-infrared spectroscopy. *Journal of the Science of Food and Agriculture,* 93**,** 238-244.

POEL, P. W., SCHIWECK, H. M. & SCHWARTZ, T. K. 1998. *Sugar Technology: Beet and Cane Sugar Manufacture*, Verlag Dr Albert Bartens KG.

PURCELL, D. E., CROFT, B. J., KOKOT, S. & O'SHEA, M. G. 2005a. Determining plant ratings without field trials- Prediction of clonal disease ratings for Fiji leaf gall using NIR spectroscopy and chemometric techniques *Australian Sugar Cane Technologists.*

PURCELL, D. E., LEONARD, G. J., O'SHEA, M. G. & KOKOT, S. 2005b. A chemometrics investigation of sugarcane plant properties based on the molecular composition of epicuticular wax. *Chemometrics and Intelligent Laboratory Systems,* 76**,** 135-147.

PURCELL, D. E., O'SHEA, M. G. & KOKOT, S. 2007. Role of chemometrics for at-field application of NIR spectroscopy to predict sugarcane clonal performance. *Chemometrics and Intelligent Laboratory Systems,* 87**,** 113-124.

PURCELL, D. E., O'SHEA, M. G. & KOKOT, S. 2009. Complex biopolymetric systems at stalk/epicuticular was plant interfaces: A near infrared spectroscopy study of the sugarcane example. *Biopolymers,* 91**,** 642-651.

PURCELL, D. E., OXLEY, J. P., COX, M. C., CROFT, B. J. & O'SHEA, M. G. 2010. Onsite rapid screening for sugarcane smut resistance using near-infrared (NIR) spectroscopy. *Australian Society of Sugar Cane Technologists.*

REEVES, I., JAMES B. 2001. Near-infrared diffuse relectance spectroscopy for the analysis of poultry manures. *Journal of Agricultural Food Chemistry,* 49**,** 2193-2197.

REEVES, I., JAMES B. & VAN KESSEL, J. S. 2000. Near-infrared spectroscopic determination of carbon, total nitrogen, and ammonium-N in dairy manures. *Journal of Dairy Science,* 83**,** 1829-1836.

REUSCH, W. 2013. *Infrared spectroscopy* [Online]. IOCD. Available: https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/Spectrpy/InfraRed/infrared.htmq [Accessed].

ROBERT, P., DEVAUX, M., QANNARI, A. & SAFAR, M. 1993. Mid and near infrared study of carbohydrates by canonical correlation analysis. *Journal of Near Infrared Spectroscopy,* 1**,** 99-108.

RODRÍGUEZ-ZÚÑIGA, U., FARINAS, C., CARNEIRO, R., DA SILVA, G., CRUZ, A., DE LIMA CAMARGO GIORDANO, R., DE CAMPOS GIORDANO, R. & DE ARRUDA RIBEIRO, M. 2014. Fast Determination of the Composition of Pretreated Sugarcane Bagasse Using Near-Infrared Spectroscopy. *BioEnergy Research,* 7**,** 1441-1453.

RUTHERFORD, R. S. & VAN STADEN, J. 1996. Towards a rapid near-infrared technique for prediction of resistance to sugarcane borerEldana saccharina walker (Lepidoptera: Pyralidae) using stalk surface wax. *Journal of Chemical Ecology* 22**,** 681-694.

SABATIER, D., THURIÈS, L., BASTIANELLI, D. & DARDENNE, P. 2012. Rapid prediction of the lignocellulosic compounds of sugarcane biomass by near infrared reflectance spectroscopy: comparing classical and independent cross-validation. *Journal of Near Infrared Spectroscopy,* 20**,** 371-385.

SCHAFFLER, K. J. & DE GAYE, M. T. D. 1997. Rapid near-infrared estimation of multi-components in mixed juice and final molasses: The possibility of day-to-day control of raw sugar factories using NIR. *Proceedings of the South African Sugar Technologists Association,* 71**,** 153-160.

SCHAFFLER, K. J., DUNSMORE, A. & MEYER, J. H. Rapid analysis of sugar products by near infrared spectroscopy. Proceedings of The South African Sugar Technologists' Association, 1993. 222-229.

SCHAFFLER, K. J. & MEYER, J. H. NIRanalysis of shreddedcane: a potential replacement for direct analysis. Proc S Afr Sug Technol Ass, 1993. 131-139.

SCHAFFLER, K. J. & MEYER, J. H. Near infra red analysis of shredded cane: A potential replacement for direct analysis of cane. Proceedings of the South African Sugar Technologists Association, 1996. 131-139.

SCHOLL, P. F., BERGANA, M. M., YAKES, B. J., XIE, Z., ZBYLUT, S., DOWNEY, G., MOSSOBA, M., JABLONSKI, J., MAGALETTA, R., HOLROYD, S. E., BUEHLER, M., QIN, J., HURST, W., LAPOINTE, J. H., ROBERTS, D., ZRYBKO, C., MACKEY, A., HOLTON, J. D., ISRAELSON, G. A., PAYNE, A., KIM, M. S., CHAO, K. & MOORE, J. C. 2017. Effects of the Adulteration Technique on the Near-Infrared Detection of Melamine in Milk Powder. *Journal of Agricultural and Food Chemistry,* 65**,** 5799-5809.

SHENK, J. S., WORKMAN, J. J. & WESTERHAUS, M. O. 2001. Application of NIR spectroscopy to agricultural products. *In:* BURNS, D. A. & CIURCZAK, E. W. (eds.) *Handbook of Near-Infrared Analysis.* New York, New York, USA: Marcel Dekker.

SIMPSON, R. & NAIDOO, Y. Progress in improving laboratory efficiencies using near infrared spectroscopy (NIRS). Proceedings of the onternational society of sugar cane technologists, 2010a. 1-9.

SIMPSON, R. & NAIDOO, Y. Using near infra red spectroscopy for rapid quantification of intermediate sugar factory products. Proceedings of the South African Sugar Technologists' Association, 2010b. 382-391.

SIMPSON, R. & OXLEY, J. Routine analysis of molasses and mixed juice by NIR spectroscopy. Proceeding of the South African Sugar Technologists' Association, 2008. 245-265.

STAUNTON, S. 2014. FOSS DA1650 Evaluation for the NIR Analysis of Mill Products. Brisbane, Australia: Sugar Research Australia.

STAUNTON, S. P., LETHBRIDGE, P. J., GRIMLEY, S. C., STREAMER, R. W., ROGERS, J. & MACKINTOSH, D. L. On-line cane analysis by near infra-red spectroscopy. Proceedings of the Australian Society of Sugar Cane Technologists, 1999. 20-27.

STAUNTON, S. P. & WARDROP, K. Development of an online bagasse analysis system using NIR spectroscopy. Proceedings of the Australian Society of Sugar Cane Technologists, 2006.

SVERZUT, C., VERMA, L. & FRENCH, A. 1987. Sugarcane analysis using near infra-red spectroscopy. *Trans Am Soc Ag Engrs,* 30**,** 255-258.

VILLATORO-PULIDO, M., MORENO ROJAS, R. E., MUÑOZ-SERRANO, A., CARDEÑOSA, V., AMARO LÓPEZ, M. Á., FONT, R. E. & DEL RÍO-CELESTINO, M. 2012. Characterization and prediction by near-infrared reflectance of mineral composition of rocket (Eruca vesicaria subsp. sativa and Eruca vesicaria subsp. vesicaria). *Journal of the Science of Food and Agriculture,* 92**,** 1331-1340.

WILLIAMS, D. H. & FLEMING, I. 1995. *Spectroscopic Methods in Organic Chemistry,* Maidenhead, Berkshire, McGraw-Hill.

WORKMAN, J. & WEYER, L. 2008. *Practical guide to interpretive near-infrared spectroscopy,* Boca Raton, CRC Press.

YAKES, B. J., BERGANA, M. M., SCHOLL, P. F., MOSSOBA, M. M., KARUNATHILAKA, S. R., ACKERMAN, L. K., HOLTON, J. D., GAO, B. & MOORE, J. C. 2017. Effects of Wet-Blending on Detection of Melamine in Spray-Dried Lactose. *Journal of Agricultural and Food Chemistry,* 65**,** 5789-5798.

8.1. Appendix 2

Lookup table to convert between wavelength (nm) and wavenumber (cm^{-1}).

nm	cm^{-1}	nm	cm^{-1}	nm	cm^{-1}	nm	cm^{-1}	nm	cm^{-1}	nm	cm^{-1}	nm	cm^{-1}	nm	cm^{-1}	nm	cm^{-1}	nm	cm^{-1}
700	14286	1000	10000	1300	7692	1600	6250	1900	5263	2200	4545	2500	4000	2800	3571	3100	3226	3400	2941	3700	2703
705	14184	1005	9950	1305	7663	1605	6231	1905	5249	2205	4535	2505	3992	2805	3565	3105	3221	3405	2937	3705	2699
710	14085	1010	9901	1310	7634	1610	6211	1910	5236	2210	4525	2510	3984	2810	3559	3110	3215	3410	2933	3710	2695
715	13986	1015	9852	1315	7605	1615	6192	1915	5222	2215	4515	2515	3976	2815	3552	3115	3210	3415	2928	3715	2692
720	13889	1020	9804	1320	7576	1620	6173	1920	5208	2220	4505	2520	3968	2820	3546	3120	3205	3420	2924	3720	2688
725	13793	1025	9756	1325	7547	1625	6154	1925	5195	2225	4494	2525	3960	2825	3540	3125	3200	3425	2920	3725	2685
730	13699	1030	9709	1330	7519	1630	6135	1930	5181	2230	4484	2530	3953	2830	3534	3130	3195	3430	2915	3730	2681
735	13605	1035	9662	1335	7491	1635	6116	1935	5168	2235	4474	2535	3945	2835	3527	3135	3190	3435	2911	3735	2677
740	13514	1040	9615	1340	7463	1640	6098	1940	5155	2240	4464	2540	3937	2840	3521	3140	3185	3440	2907	3740	2674
745	13423	1045	9569	1345	7435	1645	6079	1945	5141	2245	4454	2545	3929	2845	3515	3145	3180	3445	2903	3745	2670
750	13333	1050	9524	1350	7407	1650	6061	1950	5128	2250	4444	2550	3922	2850	3509	3150	3175	3450	2899	3750	2667
755	13245	1055	9479	1355	7380	1655	6042	1955	5115	2255	4435	2555	3914	2855	3503	3155	3170	3455	2894	3755	2663
760	13158	1060	9434	1360	7353	1660	6024	1960	5102	2260	4425	2560	3906	2860	3497	3160	3165	3460	2890	3760	2660
765	13072	1065	9390	1365	7326	1665	6006	1965	5089	2265	4415	2565	3899	2865	3490	3165	3160	3465	2886	3765	2656
770	12987	1070	9346	1370	7299	1670	5988	1970	5076	2270	4405	2570	3891	2870	3484	3170	3155	3470	2882	3770	2653
775	12903	1075	9302	1375	7273	1675	5970	1975	5063	2275	4396	2575	3883	2875	3478	3175	3150	3475	2878	3775	2649
780	12821	1080	9259	1380	7246	1680	5952	1980	5051	2280	4386	2580	3876	2880	3472	3180	3145	3480	2874	3780	2646
785	12739	1085	9217	1385	7220	1685	5935	1985	5038	2285	4376	2585	3868	2885	3466	3185	3140	3485	2869	3785	2642
790	12658	1090	9174	1390	7194	1690	5917	1990	5025	2290	4367	2590	3861	2890	3460	3190	3135	3490	2865	3790	2639
795	12579	1095	9132	1395	7168	1695	5900	1995	5013	2295	4357	2595	3854	2895	3454	3195	3130	3495	2861	3795	2635
800	12500	1100	9091	1400	7143	1700	5882	2000	5000	2300	4348	2600	3846	2900	3448	3200	3125	3500	2857	3800	2632
805	12422	1105	9050	1405	7117	1705	5865	2005	4988	2305	4338	2605	3839	2905	3442	3205	3120	3505	2853	3805	2628
810	12346	1110	9009	1410	7092	1710	5848	2010	4975	2310	4329	2610	3831	2910	3436	3210	3115	3510	2849	3810	2625
815	12270	1115	8969	1415	7067	1715	5831	2015	4963	2315	4320	2615	3824	2915	3431	3215	3110	3515	2845	3815	2621
820	12195	1120	8929	1420	7042	1720	5814	2020	4950	2320	4310	2620	3817	2920	3425	3220	3106	3520	2841	3820	2618
825	12121	1125	8889	1425	7018	1725	5797	2025	4938	2325	4301	2625	3810	2925	3419	3225	3101	3525	2837	3825	2614
830	12048	1130	8850	1430	6993	1730	5780	2030	4926	2330	4292	2630	3802	2930	3413	3230	3096	3530	2833	3830	2611
835	11976	1135	8811	1435	6969	1735	5764	2035	4914	2335	4283	2635	3795	2935	3407	3235	3091	3535	2829	3835	2608
840	11905	1140	8772	1440	6944	1740	5747	2040	4902	2340	4274	2640	3788	2940	3401	3240	3086	3540	2825	3840	2604

nm	cm^{-1}	nm	cm^{-1}	nm	cm^{-1}	nm	cm^{-1}	nm	cm^{-1}	nm	cm^{-1}	nm	cm^{-1}	nm	cm^{-1}	nm	cm^{-1}	nm	cm^{-1}
845	11834	1145	8734	1445	6920	1745	5731	2045	4890	2345	4264	2645	3781	2945	3396	3245	3082	3545	2821	3845	2601
850	11765	1150	8696	1450	6897	1750	5714	2050	4878	2350	4255	2650	3774	2950	3390	3250	3077	3550	2817	3850	2597
855	11696	1155	8658	1455	6873	1755	5698	2055	4866	2355	4246	2655	3766	2955	3384	3255	3072	3555	2813	3855	2594
860	11628	1160	8621	1460	6849	1760	5682	2060	4854	2360	4237	2660	3759	2960	3378	3260	3067	3560	2809	3860	2591
865	11561	1165	8584	1465	6826	1765	5666	2065	4843	2365	4228	2665	3752	2965	3373	3265	3063	3565	2805	3865	2587
870	11494	1170	8547	1470	6803	1770	5650	2070	4831	2370	4219	2670	3745	2970	3367	3270	3058	3570	2801	3870	2584
875	11429	1175	8511	1475	6780	1775	5634	2075	4819	2375	4211	2675	3738	2975	3361	3275	3053	3575	2797	3875	2581
880	11364	1180	8475	1480	6757	1780	5618	2080	4808	2380	4202	2680	3731	2980	3356	3280	3049	3580	2793	3880	2577
885	11299	1185	8439	1485	6734	1785	5602	2085	4796	2385	4193	2685	3724	2985	3350	3285	3044	3585	2789	3885	2574
890	11236	1190	8403	1490	6711	1790	5587	2090	4785	2390	4184	2690	3717	2990	3344	3290	3040	3590	2786	3890	2571
895	11173	1195	8368	1495	6689	1795	5571	2095	4773	2395	4175	2695	3711	2995	3339	3295	3035	3595	2782	3895	2567
900	11111	1200	8333	1500	6667	1800	5556	2100	4762	2400	4167	2700	3704	3000	3333	3300	3030	3600	2778	3900	2564
905	11050	1205	8299	1505	6645	1805	5540	2105	4751	2405	4158	2705	3697	3005	3328	3305	3026	3605	2774	3905	2561
910	10989	1210	8264	1510	6623	1810	5525	2110	4739	2410	4149	2710	3690	3010	3322	3310	3021	3610	2770	3910	2558
915	10929	1215	8230	1515	6601	1815	5510	2115	4728	2415	4141	2715	3683	3015	3317	3315	3017	3615	2766	3915	2554
920	10870	1220	8197	1520	6579	1820	5495	2120	4717	2420	4132	2720	3676	3020	3311	3320	3012	3620	2762	3920	2551
925	10811	1225	8163	1525	6557	1825	5479	2125	4706	2425	4124	2725	3670	3025	3306	3325	3008	3625	2759	3925	2548
930	10753	1230	8130	1530	6536	1830	5464	2130	4695	2430	4115	2730	3663	3030	3300	3330	3003	3630	2755	3930	2545
935	10695	1235	8097	1535	6515	1835	5450	2135	4684	2435	4107	2735	3656	3035	3295	3335	2999	3635	2751	3935	2541
940	10638	1240	8065	1540	6494	1840	5435	2140	4673	2440	4098	2740	3650	3040	3289	3340	2994	3640	2747	3940	2538
945	10582	1245	8032	1545	6472	1845	5420	2145	4662	2445	4090	2745	3643	3045	3284	3345	2990	3645	2743	3945	2535
950	10526	1250	8000	1550	6452	1850	5405	2150	4651	2450	4082	2750	3636	3050	3279	3350	2985	3650	2740	3950	2532
955	10471	1255	7968	1555	6431	1855	5391	2155	4640	2455	4073	2755	3630	3055	3273	3355	2981	3655	2736	3955	2528
960	10417	1260	7937	1560	6410	1860	5376	2160	4630	2460	4065	2760	3623	3060	3268	3360	2976	3660	2732	3960	2525
965	10363	1265	7905	1565	6390	1865	5362	2165	4619	2465	4057	2765	3617	3065	3263	3365	2972	3665	2729	3965	2522
970	10309	1270	7874	1570	6369	1870	5348	2170	4608	2470	4049	2770	3610	3070	3257	3370	2967	3670	2725	3970	2519
975	10256	1275	7843	1575	6349	1875	5333	2175	4598	2475	4040	2775	3604	3075	3252	3375	2963	3675	2721	3975	2516
980	10204	1280	7813	1580	6329	1880	5319	2180	4587	2480	4032	2780	3597	3080	3247	3380	2959	3680	2717	3980	2513
985	10152	1285	7782	1585	6309	1885	5305	2185	4577	2485	4024	2785	3591	3085	3241	3385	2954	3685	2714	3985	2509
990	10101	1290	7752	1590	6289	1890	5291	2190	4566	2490	4016	2790	3584	3090	3236	3390	2950	3690	2710	3990	2506
995	10050	1295	7722	1595	6270	1895	5277	2195	4556	2495	4008	2795	3578	3095	3231	3395	2946	3695	2706	3995	2503