Predicting Nutritional Content of Native Forage Feed using ATR-FTIR and NIR Chemometrics

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Abstract

Near infrared (NIR) reflectance spectroscopy has historically dominated the agriculture industry in the prediction of the nutritional content of pasture and forage in New Zealand. This study investigates using an alternative infrared reflectance technique in the mid infrared (MIR) region, Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy, and compares it to NIR, for the prediction of the chemical composition of native forage feed for sheep. Six native forage species and one non-native control species comprised 181 samples, which were recorded with both NIR and ATR-FTIR spectroscopy. Spectral pre-treatment was applied to all spectra in the form of a first-order Savitzky-Golay (SG) smoothening filter. Prediction of nutritional content for six analytes was achieved for both IR methods, using Principal Component Analysis (PCA) and a Partial Least Squares (PLS) regression model. The predictive ability of ATR-FTIR and NIR models was evaluated using the coefficient of determination (R^2), Root Mean Square Error of Cross Validation (RMSECV), and Relative Performance Deviation (RPD). NIR had superior R^2 and RPD, and similar RMSECV to ATR-FTIR for all analyte predictions. The best models were crude protein (CP) for NIR (R^2: 0.95, RPD: 5.58) and metabolisable energy (ME) for FTIR (R^2:0.79, RPD: 3.52). Post prediction statistics were also investigated for FTIR and NIR, finding that a ‘one size fits all’ blanket model for all species and tissue types was sufficient for quality prediction of CP, ME, and neutral detergent fiber (NDF) for native shrub species. These models suggested that ME and NDF predictions were similar between NIR and FTIR but NIR was superior to FTIR for CP. Overall, this study demonstrates the considerable potential of ATR-FTIR for quality nutritional content predictions, that are comparable to NIR.

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Firstly, I must express my thanks to my supervisors Professor Mark Waterland, Professor Paul Kenyon and Professor Patrick Morel. Thank you for sharing your knowledge and expertise with me and making time available to meet throughout the project. Your constant support and patience were more than I could ask for. I need to say a special thanks to Mark for the countless hours spent with me in his office developing Python code, fixing code errors, and explaining theory. Paul and Patrick, your knowledge of nutrition, forage, and statistics is unmatched whilst also providing amazing proofreading and editorial skills. This thesis could not have been completed without all your dedication and enthusiasm, thank you.

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Abbreviations

R^2	Coefficient of Determination
MSE	Mean Square Error
RPD	Relative Performance Deviation
ATR-FTIR	Attenuated Total Reflectance Fourier Transform Infrared
NIR	Near Infrared
MIR	Mid Infrared
Calib	Calibration
Pred	Prediction
NDF	Neutral Detergent Fiber
ME	Metabolisable Energy
CP	Crude Protein
DOMD	Dry Organic Matter Digestibility
ADF	Acid Detergent Fiber
CV	Cross-Validation
SG	Savitzky-Golay
PLS	Partial Least Squares
PCR	Principal Component Regression
PCA	Principal Component Analysis
RMSECV	Root Mean Square Error of Cross Validation
CCC	Concordance Correlation Coefficient
MPE	Mean Performance Error
RPE	Relative Performance Error

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1.0 Introduction

Determining the nutritional content of forage feed is significant to farmers, as the growth and performance of livestock are determined by forage quality. Knowing the nutritional content of forage is important to balance the nutritional requirements of livestock, as well as optimising favourable nutritional components for consumption (Monaghan et al, 2007).

In New Zealand, feed nutritional values and ruminant animal requirements are expressed in terms of Metabolisable Energy (ME; Nicol and Brookes, 2017), and Metabolisable Protein (MP, Brookes and Nicole, 2017). ME can be determined in animal trials but this is complicated as faeces, urine and gas (mainly methane) need to be measured. Thus, an in vitro assay measuring the digestibility of the organic matter (DOMD) is commonly used to estimate ME (Roughan and Holland, 1977). No values are currently available on the MP content of native shrubs. However, as MP is mainly determined by the crude protein concentration in the feed, CP will be used instead of MP. Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) were also investigated in this study, as the fibre content is related to both the digestibility of the feed and amount of feed that an animal can eat. Therefore, in this study six analytes were investigated: Dietary Crude Protein (CP), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Metabolisable Energy (ME), In Vitro Dry Organic Matter Digestibility (In Vitro DOMD) and Ash. These nutritional components have been selected as they are significant for farmers around the globe and therefore, appear in a wide range of previous research.

Recently, there has been increased interest in using native shrubs as alternative forages for sheep on hill farms. With very little and in some cases no nutritional data available in the literature, very costly wet chemical analysis is the only way to fill in the missing gaps in the literature. Due to the cost involved with wet chemical analysis and the drive for future research, there is a demand for a significantly cheaper and quicker method to obtain nutritional content. A common alternative method uses NIR spectroscopy with available wet chemical data to form a prediction calibration. More recently, ATR-FTIR spectroscopy has shown the potential to produce similar prediction calibrations (Cleland et al, 2018). Neither of these methods have been used for New Zealand native shrubs previously, so a prediction calibration and prediction result for both methods will massively add to the literature. More than this, a comparison between NIR and ATR-FTIR predictions can be compared.

There has been limited research conducted using forage native to New Zealand. The nutritional content of native species has only been recorded with wet chemical methods and there are many gaps in the literature. This research will use six native species including Hoheria populnea (Houhere or lacebark), Griselinia littoralis (Kapuka or NZ broadleaf), Pittosporum crassifolium (Karo), Coprosma robusta (Karamu), Pseudopanax arboreus (Puahou or Five finger) and Melicytus ramiflorus (Mahoe or whiteywood). Each of these species is selected due to availability and palatability to animals. Salix kinuyanagi (Willow) was used as a control species for this study, already being used as supplementary herbage and has been researched many times before (Oppong et al, 2001; Kemp et al, 2001). Ryegrass and White clover nutritional values were included in table 2, to easily compare popular pasture to the native forage species.

The current gold standard and most popular method of determining nutritional content is the wet-chemical analysis, based on highly optimised chemical principles involving several techniques to determine the desired analyte (Stokes & Prostko, 1998). This, however, takes significant time and money to complete and is sensitive to good sampling techniques and procedures in the lab (Schroeder, 2004). As a result, near-infrared (NIR) spectroscopy has been developed over many years, with the use of computer modelling and machine learning, to predict chemical values which are used in the determination of nutritional content (Rukundo et al, 2021; Parrini et al, 2018; Norris et al, 1976). NIR is faster, cheaper and maintains accurate predictions for nutritional information compared with the gold standard wet chemical method (McLellan et al, 1991).

Since then, developments within the MIR region have come about with the addition of the ATR attachment, allowing sample thickness to significantly increase from the previous few microns thick. As a result, ATR-FTIR spectroscopy has been used in a similar way to the well-established NIR spectroscopy for predicting the nutritional content of pasture and forage.

MIR spectra, cover signals which come from functional groups and the fingerprint region (4000-400cm-1). As a result, these chemical signals can be used in the identification of nutritional components within recorded spectra (Stokes & Prostko, 1998; Cleland et al, 2018). NIR produces spectra with high vibrational frequencies, many overtones, bad sensitivity, and the spectra range provides little amounts of chemical information (Alomar et al, 2003). This may suggest MIR spectra have a more powerful predictive power than NIR and could even compare to the gold standard wet-chemical method for determining nutrient content. While there has been research conducted on native forage in New Zealand, nutritional values have only been found using wet chemistry methods. Currently, there is a gap in the knowledge concerning alternative prediction methods of native forage species in New Zealand. Both ATR-FTIR and NIR prediction using chemometrics have not been investigated. This research will determine if MIR and NIR can be used to predict the nutritional content of native forage feed using chemometrics. A primary research question looking to be answered is how well ATR-FTIR predictions compare to NIR at predicting the nutritional content (how close to the wet chemistry results can they predict), as well as the performance of the models concerning external validation and Relative Performance Deviation (RPD).

This research will be significant as it will be the first to predict the nutritional content of native forage using ATR-FTIR and NIR Chemometrics, whilst also being a comparison between techniques and the wet chemical gold standard. This both helps develop new techniques for modern farming development and promotes the use of native supplementary forage species in New Zealand.

2.0 Literature Review

2.1 Forage

Nutritional Components of importance in a sheep's diet

A sheep requires proper nutrition to fuel and maintain its body. There are several significant nutritional components that have major importance in producing high-quality sheep. The following includes six important nutritional components of quality forage. If balanced correctly, these components will support high-quality sheep.

2.1.1 Analytes

Crude Protein

Dietary Crude Protein (CP) provides the protein needed for the synthesis of wool, hormones, body tissue and milk. CP is made up of nitrogen (N) containing compounds and true protein. CP is calculated by N*6.25=CP (Mariotti et al, 2008).

Neutral Detergent Fiber

Neutral Detergent Fiber (NDF) contains both digestible components cellulose and hemicellulose, as well as indigestible lignin. This nutrient component is a measure of fiber content and links to the bulkiness of the feed which helps determine its digestibility.

Acid Detergent Fiber

Acid Detergent Fiber (ADF) is similar to NDF helping determine the digestibility of a feed but only contains the less digestible cellulose and lignin. The higher the ADF the lower the digestibility, typically resulting in a lower energy also.

Metabolisable energy

Metabolisable energy (ME) is the net energy that can be used by an animal for metabolic purposes such as maintenance, growth, lactation, activity and pregnancy. ME is an estimate, predicted through the digestibility of the feed. Figures 1&2 clearly display this.

Ash

Ash accounts for all inorganic matter within the feed, giving the total mineral proportion.

In Vitro Dry Organic Matter Digestibility

In Vitro Dry Organic Matter Digestibility (In Vitro DOMD) is the proportion of organic matter (dry matter DM) that can be absorbed in the ruminant's digestive tract. This is calculated as DM% and is useful in determining ME and CP (Al-Arif et al, 2017).

The relationship between chemical components can be very complex. Figure 3 illustrates the basic relationship of several components.

Under NZ pastural conditions the ME of herbage is more likely limiting than protein percentage (Hynd, 2019, p.135). Most herbage in NZ has an adequate protein percentage. Energy is used by the animal to support its maintenance and other physiological needs (Figure 1,2&6). It is known that as ME MJ/kg Dry Matter (DM) go below 10.5MJ/kg DM, due to its impact on efficiency of digestibility, ME requirements for maintenance and physiological needs increase.

Animal performance is driven by feed intake (Hynd, 2019; Rattray, Brookes, & Nicol, 2017) in terms of both total intake and the quality of the herbage. Therefore, nutritional traits such as NDF and digestibility influence intake and thus performance (figure 4,5).

figure 4 displays the ME and CP requirements for different levels of animal production. High production is achieved when ME is in the range of 11-13 MJ/kg DM and CP of 22-

28%, whilst moderate production occurs at 9-13 MJ/kg DM ME and 16-22% CP (Hynd, 2019).

The quality of herbage (ME MJ/kg DM) impacts the ME utilization requirements of an animal. A high herbage (ie >11 MJ/ME/ewe/day) will produce high performance ewes that can support larger live weight (Table 1, Rattray, Brookes, & Nicol, 2017).

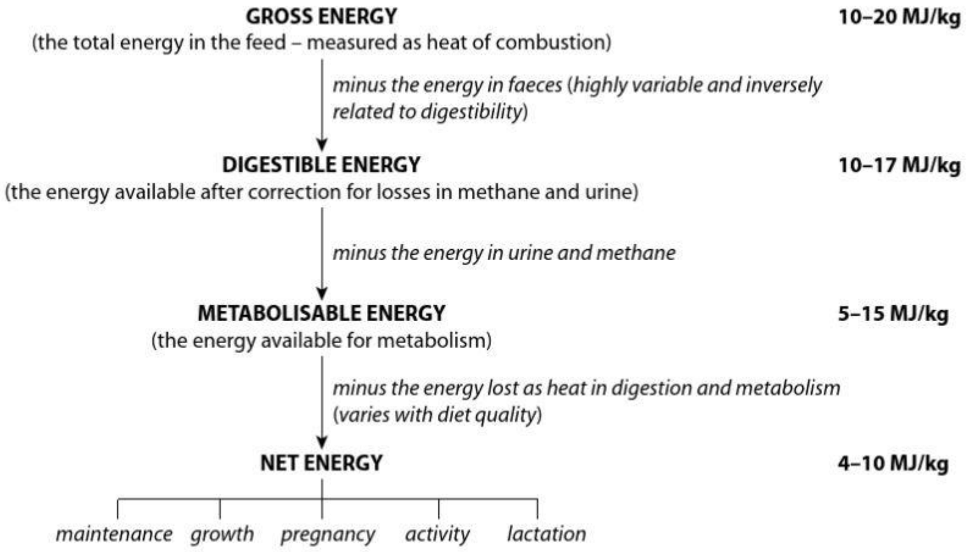


Figure 1: Diagram representing energy breakdown during digestion (Rattray, Brookes, & Nicol, 2017, p.51).

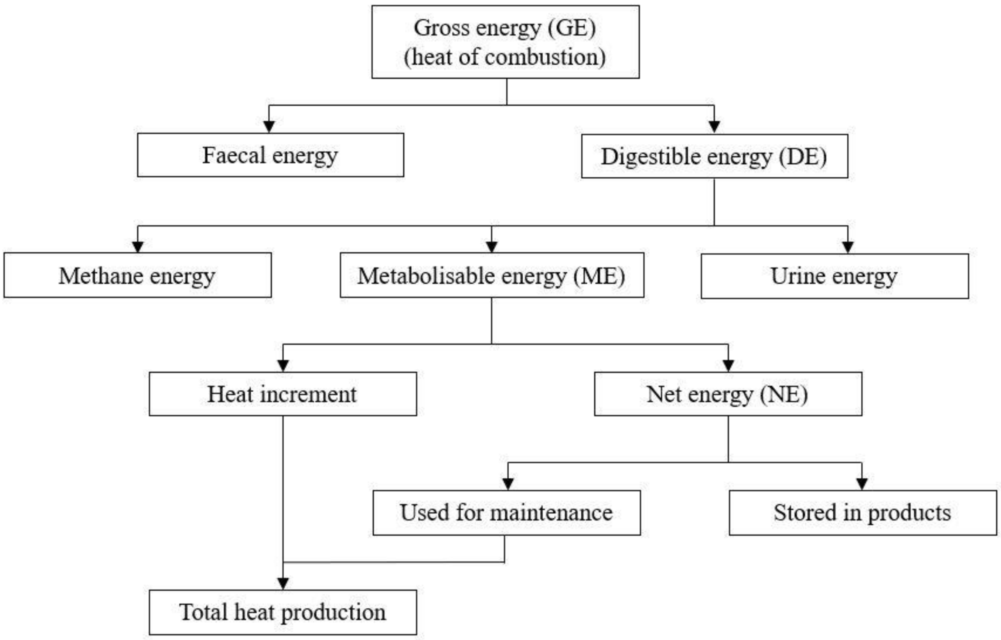


Figure 2: Breakdown of animal feed energy with their typical energy values (Hynd, 2019, p.14).

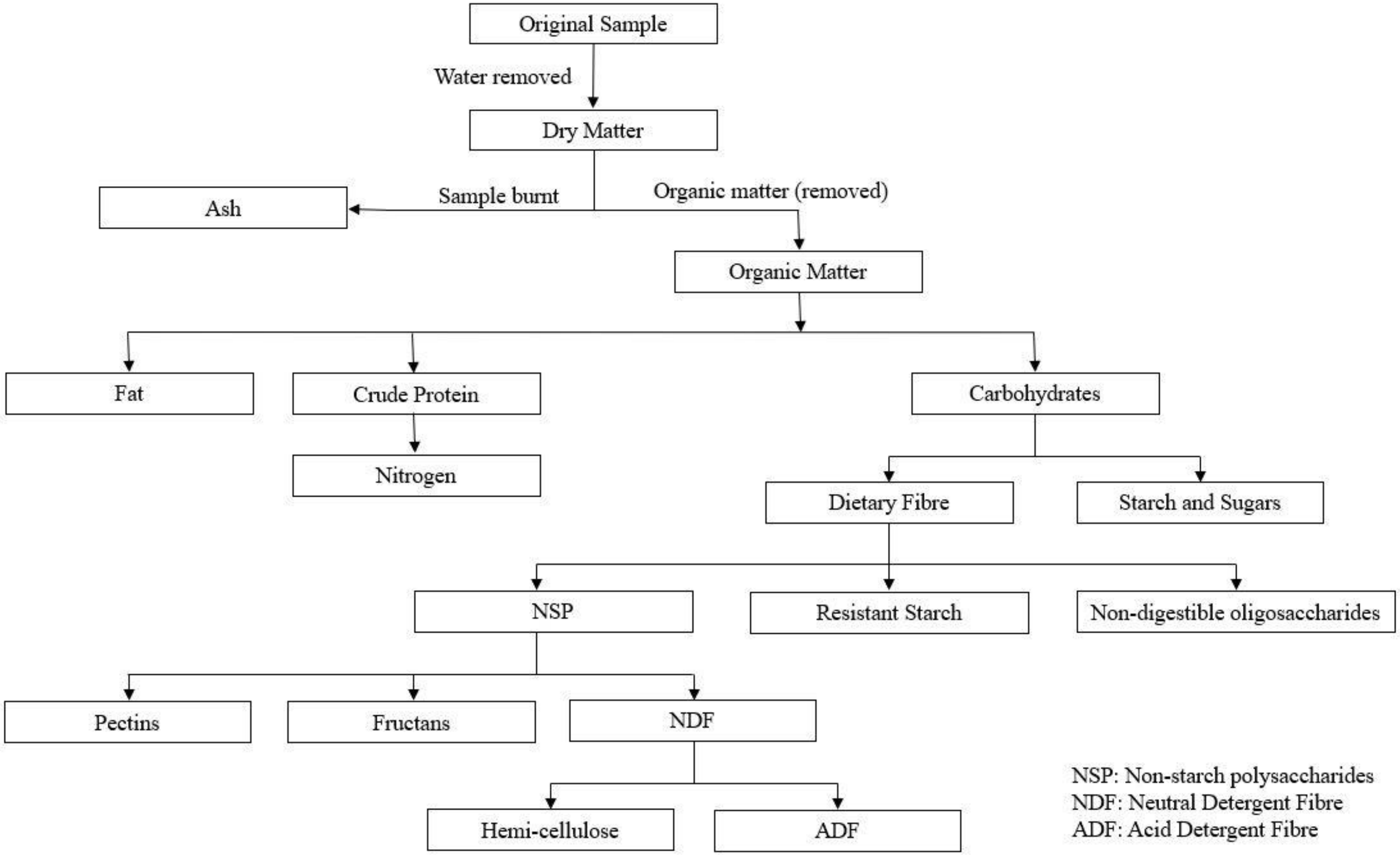


Figure 3: Diagram showing the relationship between chemical components in forage.

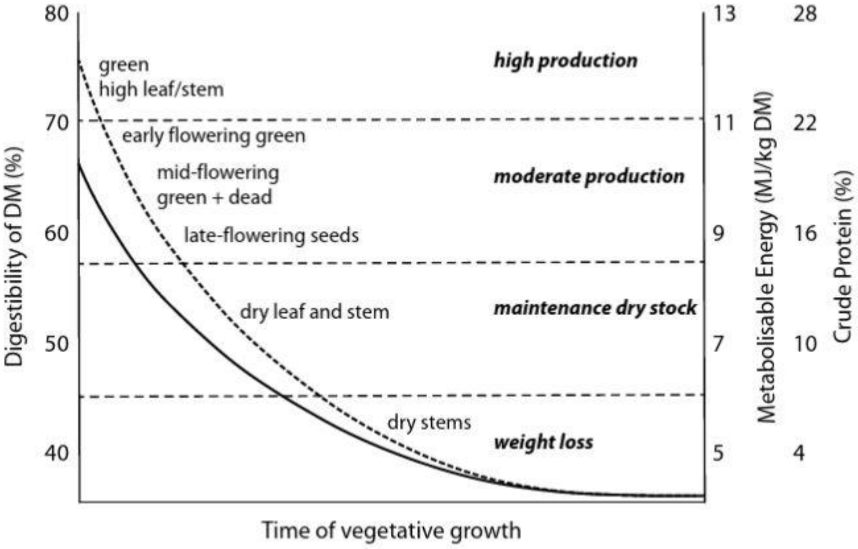


Figure 4: Nutritive value changes occurring to tropical (solid line) and temperate (dashed line) plants throughout seasonal changes (Hynd, 2019, p.135).

As NDF increases past 33 % DM VFI decreases because of increased retention time to feed in the rumen due to low digestibility. This illustrates the importance of managing NDF% in forage for ruminants (Hynd, 2019).

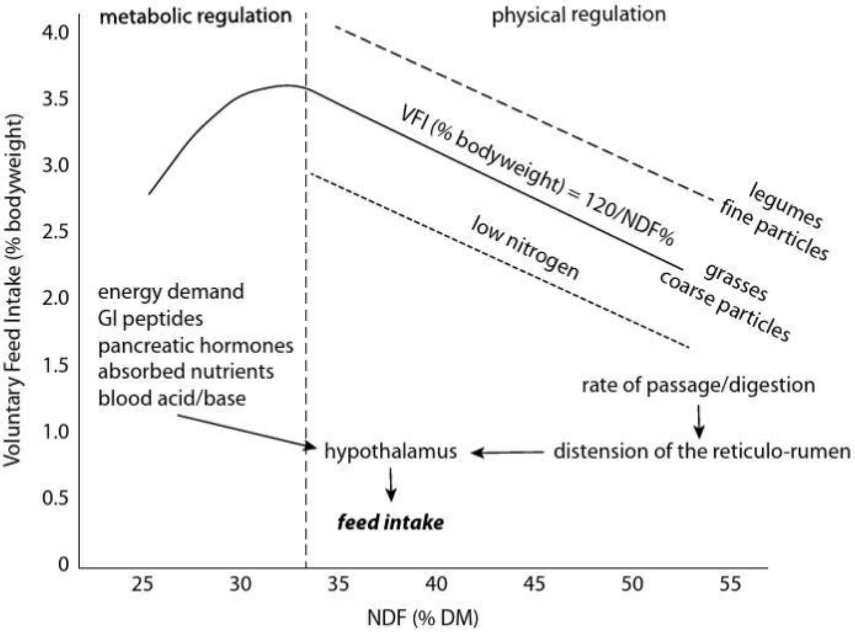


Figure 5:The relationship between NDF and voluntary feed intake (VFI) (body weight %) in ruminants.

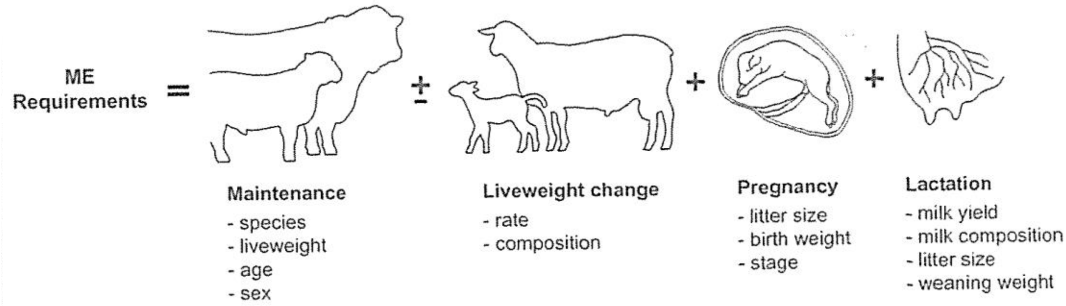


Figure 6: Diagram illustrating the components involved in calculating metabolisable energy.

All these components can be accurately predicted. The Australian (CSIRO, 2007), French (INRA, 1989) and NZSAP are the most common methods (Rattray, Brookes, & Nicol, 2017).

Table 1: Maintenance requirement of adult ewes for metabolisable energy (Rattray, Brookes, & Nicol, 2017, p.154).

	Live weight (kg)				
Class	40	50	60	70	80
	MJ ME/ewe/day				
Flat land			9.0	10.0	11.0
Rolling/easy hill		8.0	10.0	11.0	
Hard hill	7.5	9.0	11.0		

Notes: Add/*subtract* 7% per MJ ME for diets below/*above* 10.5MJ ME/kg DM.

2.1.2 Native Forage

2.1.2.1 Ryegrass (rye) and White clover (wc) sword mix.

This species mix is the most sown pasture in New Zealand and therefore in cultivated land the most grazed pasture mix. In addition, ryegrass itself is the most sown species. Therefore, in the following sections due to the lack of data on native shrub species, the available data allows for a comparison between ryegrass and rye/wc mix with native forage.

2.1.2.2 Salix kinuyanagi (Willow).

This species was selected as it is already in use as supplementary herbage on some farms in New Zealand (Hathaway 1986; McCabe and Barry 1988; Oppong et al 2001). Salix is also utilised as a worldwide feed source. There is well established data on the species making it a good comparative species of tree (Pitta, 2007). The nutritive values are presented in table 3.

2.1.2.3 Hoheria populnea (Houhere or lacebark).

There has been little study conducted as to whether Hoheria will be suitable as a forage feed for animals (Carnachan, 2018). Although this species has not been widely researched for livestock, a very similar species coming from the same family of Malvoideae, called *Lavatera plebian* has been used as herbage in Australia (Mitchell, 1982). Another similar species in New Zealand, *Hoheria glabra* was found to be browsed on by deer (Forsyth, Coomes, & Nugent 2003). Table 3 indicates the nutritional composition of this species appears to have been studied by three authors (Sims et al, 2018; Simmonds, 2020; Wangui, 2023). The crude protein data appears to be lower than white clover compared with ryegrass alone and with rye/wc mix, while ME and NDF were similar. Compared to Salix, Hoheria has similar CP, ME and NDF.

2.1.2.4 Griselinia littoralis.(Kapuka or NZ broadleaf)

This species of native shrub has been recorded to be preferred by deer and goats in New Zealand forests (Nugent et al, 1997; Forsyth, Richardson, and Menchenton, 2005; Nugent, 1990).This species has relatively low levels of nitrogen in the leaves compared to the other short angiosperms such as *Coprosma lucida, Coprosma foetidissima and Neopanax* colensoi, but average phosphorus levels (Coomes et al, 2009). Bee et al, (2011) found there to be low concentrations of foliar lignin. The CP is lower than white clover, ryegrass and rye/wc mix but similar to Salix, while the ME and NDF were similar in all white clover, ryegrass, rye/wc mix and Salix observed in table 2.

2.1.2.5 Pittosporum crassifolium (Karo)

Limited research has been conducted on this species with only Simmonds (2020) and Wangui (2023) appearing to have produced nutritional data. Kardol et al, (2014), have reported that this shrub is browsed by ungulates in the wild. Nutritional content data in Table 3 shows similar values of NDF, ME and CP to Salix. White clover, ryegrass and rye/wc mix also had similar values for NDF and ME however a higher value for CP.

2.1.2.6 Coprosma robusta (Karamu)

Coprosma robusta is one of the most common species of native plant in New Zealand (Orwin, 2007). Although the nutritional content is not well researched it appears to have good palatability for stock (Dodd et al, 2007). Compared to pasture in New Zealand, C. robusta has been reported to have low nitrogen and phosphorus concentrations (Hahner, 2012). While Franklin (2014), reported comparable nitrogen and phosphors to pasture. CP of Coprosma is lower than white clover, ryegrass and rye/wc mix but similar to Salix. The ME and NDF of white clover, ryegrass, rye/wc mix and Salix are similar to the values of Coprosma in table 3.

2.1.2.7 Pseudopanax arboreus (Puahou or Five finger)

This species is common in forest environments and has been identified to have high palatability for ungulates and is a major component of deer diets (Bulloch, 1995; Forsyth et al, 2002). Fibre and crude protein were found at 27% and 8% respectively, only half of what was found in Melicytus ramiflorus (Fitzgerald, 1997). Enright and Ogden (1987) reported average levels of both nitrogen and phosphorous. Simmonds (2020) reports similar ME with white clover, ryegrass, rye/wc mix and Salix with a lower value for NDF and CP. Fitzgerald (1976) reported a similar CP to Salix but higher (almost double) ME values.

2.1.2.8 Melicytus ramiflorus (Mahoe or whiteywood)

Mahoe is widespread across New Zealand and is a preference for deer possum and chamois showing high palatability (Forsyth et al, 2002; Yockney & Hickling 2000). In contrast Bulloch (1995), and Greenwood and Atkinson (1977), found Mahoe to be only partially palatable with a toxic secondary compound being produced, lowering its palatability. The reported ME from Fitzgerald (1976) was higher than all values in table X, white clover, ryegrass, rye/wc mix and salix. CP was lower than white clover but showed similar values to ryegrass and white clover, whereas the value of Salix was almost half that of Mahoe. No NDF values were reported for Mahoe.

Table 2: Nutritional values of non-native species that have common international use. (L=Leaf sample, S=Stem sample).

Species	References	CP %	ME MJ/kg	Ash %	NDF %	ADF %	In Vitro DOMD%
White clover	Burke et al, (2000)	27			26		
	Fulkerson et al, (2007)	24	10.0		28	21	
	Lindsay et al, (2007)	28	11.7		26		
	Fraser & Rowarth (1996)	28					
Perennial Ryegrass	Muir et al, (2020)	9.6	9.6		57.1	29.1	
	Golding et al, (2011)	20	9		48		
White clover/ Ryegrass mix	Somasiri et al, (2016)	24.7-26.3	10.4		41.8-43.0		
	Ekanayake et al, (2020)	12-16.5	10.6-11		28.7-32.7	22.9-26.2	
S. kinuyanagi	Oppong (1998)			5.9-6.5	35.7-38.7		
	Kemp, Barry, & Douglas, (2003)	7.1	9.7	1.6	38.1	26.4	
	Simmonds (2020)	8.8L 4.0S	10.5L 10.0S	5.7L 4.1S	48.7L 52.8S	34.1L 40.0S	63.7L 60.3S

Table 3: Native forage species nutritional vales within the literature. (L=Leaf sample, S=Stem sample).

Species	References	CP %	ME MJ/kg	Ash %	NDF %	ADF %	In Vitro DOMD%
H. populnea	Sims et al, (2018)			12.1			
	Simmonds (2020)	11.6L 7.4S	10.8L 9.2S	12.2L 9.7S	35.5L 53.1S	19.4L 42.2S	65.6L 54.9S
	Wangui (2023)	14.0L 8.3S	11.4L 9.6S	11.6L 9.0S	37.6L 53.5S	20.2L 41.5S	71.2L 60.0S
G. littoralis	Kurokawa et al, (2010)					26.4	
	Simmonds (2020)	6.0L 3.4S	11.8L 9.8S	6.9L 5.0S	30.6L 49.5S	21.2L 39.9S	71.5L 59.0S
	Wangui (2023)	6.2L 4.2S	11.9L 10.0S	7.3L 5.6S	32.1L 48.6S	22.3L 38.6S	74.6L 62.5S
P. crassifolium	Wardle et al, (2009)					28.9	
	Simmonds (2020)	6.6L 4.9S	10.9L 9.9S	8.2L 8.6S	34.8L 46.2S	23.3L 35.6S	66.9L 59.0S
	Wangui (2023)	6.2L 4.2S	12.0L 9.6S	6.5L 6.7S	36.4L 52.0S	20.7L 41.0S	74.7L 60.0S
C. robusta	Kurokawa et al, (2010)					29.7	
	Simmonds (2020)	7.8L 4.5S	11.6L 10.1S	7.5L 6.1S	34.7L 46.1S	24.1L 35.5S	70.3L 61.2S
	Wangui (2023)	7.9L 4.7S	12.0L 10.2S	7.0L 6.4S	37.4L 46.6S	22.4L 36.7S	75.0L 63.7S
P. arboreus	Kurokawa et al, (2010)					23.0	
	Fitzgerald, (1976)	8.6	21.6	5.7			
	Simmonds (2020)	5.4L 2.6S	12.2L 10.8S	6.3L 7.6S	21.0L 35.4S	15.0L 27.9S	73.8L 64.1S
M. ramiflorus	Fitzgerald (1976)	14.5	17.8-18.8	9.7			
	Wardle et al, (2009)					34.2	
	Kurokawa et al, (2010)					30.0	

The important herbage characteristics regarding sheep are well characterised (Hynd, 2019; Rattray, Brookes, and Nicol, 2017). Traditional methods to resolve these herbages include wet chemistry and NIR. However, these can be slow and expensive (Chen et al, 2015; Meder et al, 2007; Parrini et al, 2019). More recently it has been shown by Cleland et al, (2018), that ATR-FTIR can be successfully used to resolve nutrient herbage components in ryegrass-white clover and herb-clover mixes. It is unknown if this technique would be successful for native shrubs.

Therefore, this research was conducted to identify whether ATR-FTIR can be used to predict the nutritional content of New Zealand native forage and if so, whether is it comparable to the NIR method used as the alternative to the gold standard wet chemistry method.

2.1.3 Use of MIR and NIR within the literature

Corson et al, (1999) provides a detailed insight into how NIRS analysis is used and the pros and cons of using this method developed for the New Zealand dairy industry, to predict forage chemical composition. Corson suggests that this method has provided an alternative to exclusively using wet chemistry methods. However, that data is still required to develop more accurate calibrations based on a large dataset. The NIRS data was recorded across 1100nm-2500nm wavelength producing chemical bond signals CH2, OH, NH and SH groups. The overall sensitivity and signal strength are dependent on good samples to produce a good spectrum. Both wet-chem and NIRS data must be good for the calibration, of which PCA is the basis, with a first derivative PLS interpretation. The prediction developed had high predictive capability for CP and NDF with $R^2>0.95$, whilst other components ranged from $R^2=0.67$-0.90. Overall, this paper demonstrated that NIRS could predict nutritional content well however, it was heavily determined by strong wet-chem data to make a good calibration and prediction (Corson et al, 1999).

Yang et al, (2017) predicted the forage quality of Italian ryegrass NIRS. Yang was most interested in making prediction models for CP, acid detergent fiber (ADF), NDF, and water-soluble carbohydrates (WSC). Their results showed a slightly larger range of content was obtained when compared to previous studies however, the critical wavelengths occurred in the same regions. The Kennard-Stone algorithm was used to select 123 samples of a larger sample pool which were used to make the first analysis model using OPUS software. PLS regression was conducted to correlate the NIRS spectra and the wet-chem data. Unlike the paper above, this paper used pre-treatments of which FD+MSC PT provided the best linear result and was used in the calibration. The CP turned out to have the best fit with the lowest RMSECV and highest R^2. All components fell within the expected ranges for Italian ryegrass. This calibration was then tested with an external validation which produced linear plots with satisfactory accuracy R^2 and RPD values, 0.91, 3.2 for CP, 0.89, 3.1 for ADF and 0.88, 3.0 for NDF. Therefore, these three models were successful and provided strong predictions (Chen et al, 2015).

Cleland et al, (2018) display that MIR, ATR-FTIR spectroscopy is a viable route for predicting chemical components of oven dried temperate forage feed, using a specially modified PLSR model. This paper is a breakthrough in the current literature as there are not many predictions for the nutritional content of forage using MIR spectroscopy. After the calibration and cross-validation, the model underwent external validation involving a dataset which the model was not made from. There was significant processing with several different pre-treatments investigated of which a first order Savitzky-Golay (SG) treatment was favoured and produced accurate predictions, comparable to NIR results for

NDF (R^2: 0.86, RPD: 2.60) and hemicellulose (R^2: 0.86, RPD: 2.60). The other analytes being predicted were lower but are still useful for predictions of protein (R^2: 0.72, RPD: 2.33), ADF (R^2: 0.70, RPD: 1.79), DOMD (R^2: 0.72, RPD: 1.62) and ME (R^2: 0.72, RPD: 1.67). A good model is RPD>2.0 and a fair model is 1.4<RPD<2.0 (Cheng et al, 2019; Mammadov et al, 2020; Paltseva et al, 2022)). Therefore, NDF, hemicellulose and protein are good and the rest are fair.

A communication paper in 2016 summarised how a handheld portable NIR machine was used to predict NDF, CP and DM with moderate accuracy, R^2: 0.82, 0.71 and 0.86, for farm forage by Berzaghi et al, (2005). A prediction model was developed using more than 300 samples, using new samples for the prediction. The lower R^2 for CP was believed to be due to the limited variability of CP within maize silage (Berzaghi et al, 2005).

Another paper by Monrroy et al, (2017) investigated NIR predictions on NDF as well as ADF, cellulose and CP, with the best predictions employing PLS. 153 samples of *Brachiaria* spp were split into the calibration set (123) and external validation set (30) used for the prediction. Both NDF and ADF produced good results, R^2: 0.86 and 0.9, unlike the other two, cellulose and CP producing poor results, R^2: 0.73, 0.53. CP was also the lowest in the calibration set, 0.65 compared to 0.87-0.9 R^2 for the other constituents. Monrroy et al, (2017) reason that because of the narrow range of CP concentrations that were used for calibration, there was a difference in experiential and estimated results. The models had good predictive ability for NDF, ADF, cellulose and CP with RPDs of 3.4, 5, 2.6 and 3.5 respectively. This demonstrated a high R^2 does not indicate poor prediction ability of a model. In contrast, Asekova et al, (2016) found NIR prediction models for NDF and ADF had lower performance compared to CP and crude fat (CF) in soybean samples. 353 soybean samples were recorded and used for the calibration of models, implementing modified PLS and principal component regression (PCR). Prediction via a validation set achieved high R^2 for CP and CF, 0.91 and 0.93, good for NDF, 0.77 and low for ADF, 0.5. The evaluation of the calibration by RPD showed successful results for CP and CF, 3.25 and 3.85, and moderately useful calibration of NDF and ADF 2.07 and 1.97. Asekova et al, (2016) suggested the lower NDF and ADF could be caused by the negative correlations with protein and oil content.

Hell et al, (2016) compared the performance of MIR (ATR-FTIR) and NIR spectroscopy for the nutritional predictions of wheat bran. The findings showed for individual analyte models, protein was better predicted with MIR than NIR, R^2: 0.75, 0.66 respectively. Ash (R^2: 0.85, 0.91) and every other analyte tested (5) showed better prediction for NIR over MIR. When testing the methods over a universal processing method, there was a distinct separation of predictive ability with NIR producing higher R^2 and lower error across all analytes, suggesting its superior robustness. Hell et al, (2016) summarise MIR is more error prone for the one size fits all due to relying on small spectral ranges of characteristic bands in multivariate calibration. Even so, it was concluded either method would be suitable as an alternative rapid analysis to wet chemical analysis, with NIR showing superiority over MIR with the current calibrations. A study by Shi, Li and Yu, (2019) concluded NIR performed slightly better than MIR for the prediction of CP and intestinal protein digestibility (IPD) of wheat. 48 wheat samples were processed with 10 preprocessing algorithms and then calibrated using the PLSR method. The results were excellent for CP with NIR and MIR models producing high R^2 (0.98, 0.96) and RPD (8.0, 4.9) values. IPD only showed approximate quantitative prediction with R^2 and RPD of 0.68 and 1.83 for NIR, and 0.67 and 1.79 for MIR. Shi et al, (2019) suggest two factors that could influence the performance of MIR predictions related to interferences correlated to particle size and the homogeneity of the samples (Brás et al, 2005).

Richardson and Reeves, (2005) are one of the few examples comparing NIR and MIR predictions as an alternative to wet chemical analysis. 72 dried conifer foliage samples are used in the PLSR calibration and prediction model for 11 analytes, including NDF and ADF. MIR R^2 for calibration was higher for 8 of 11 analytes compared with NIR. The R^2 prediction for MIR and NIR did not suggest either method was superior for NDF (0.8, 0.88) or ADF (0.91, 0.89). MIR had a larger prediction bias than NIR which could suggest the NIR method may be more robust in this instance. Richardson and Reeves, (2005) concluded that there was not enough clear evidence to put one method above another however, as MIR is still relatively primitive, further development of calibrations may see MIR become equally as good or surpass NIR in the future.

Project aim

In New Zealand, nutritional values are widely reported for many pasture and forage species using traditional methods, however, there are gaps in the literature regarding native forage species, such as Hoheria, Griselinia, Pittosporum, Coprosma, Pseudopanax and Mahoe. Within the literature, there are many examples employing infrared spectroscopy for the prediction of chemical composition, where NIR is the dominant method in the agriculture sector compared with ATR-FTIR. Currently, no calibrations or predictions have been made for native forage species with NIR or ATR-FTIR chemometrics. Therefore, this research will fill in the missing gaps of native forage nutritive values across six analytes (CP, ME, NDF, ADF, Ash and *In Vitro* DOMD) using wet chemical analysis, successfully calibrate and predict the nutritional content of native forage with both NIR and ATR-FTIR chemometrics, and finally, compare predictions and model robustness of each method. This project aims to demonstrate the potential for a newer rapid prediction method that is comparable to NIR and an alternative to traditional wet chemical analysis.

3.0 Materials and methods

This section describes the methodology used throughout this project. The first section discusses the location, and which native samples were planted and collected. The second section will describe how samples were prepared and analysed using traditional wet chemistry, ATR-FTIR and NIR spectroscopy. The next section outlines how Python was implemented in the processing, prediction of recorded spectra and evaluation of models. The final section describes the statistical analysis conducted.

3.1 Forage Feed Samples

A total of 181 native shrub samples were used for this analysis, including 28 Hoheria, 28 Griselinia, 38 Pittosporum, 38 Coprosma, 19 Pseudopanax, 10 Mahoe and 20 Salix. The samples were collected in 2020 and 2021 from Massey University farm Dairy No.4 (5km south of Palmerston North in New Zealand) making up trials TN20-102 (16), TN-20-208 (56), TN20-898 (40) and TN21-852 (69). Previous students have also used the samples from thesis trials so on occasion sample bags were left empty. In those rare cases, the NIR and ATR-FTIR spectra could not be recorded, so the wet chemistry data was removed from the dataset. Wet chemistry results from these trials can be found in Appendix 1. All samples were collected by hand picking stems and leaves, with no stems exceeding 5mm in diameter. The samples were separated into stem and leaf bags and then frozen (-20 ^{o}C) until being freeze dried. Once freeze dried, the collected samples were ground using a grinding machine with a 1mm size screen attached. The ground contents were transferred into labelled bags and were ready for analysis. Although the 1mm screen was used, larger particle size was also present in each bag.

3.2 Traditional Chemical Composition (wet chemistry)

The Massey University Nutrition laboratory conducted both the freeze drying grinding and nutritional analysis of all stem and leaf samples. The analysis included Nitrogen, dry matter (DM), Ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), digestible organic matter content (DOMD), metabolisable energy (ME = 0.16× DOMD) and crude protein (CP= nitrogen x 6.25). Nitrogen (N) and CP were determined using the Dumas total combustion method (968.06, Association of Official Analytical Chemists (AOAC), 1990, Mariotti et al, 2008). DM was determined using AOAC methods 930.16 and 925.10 (AOAC, 1990). Ash using AOAC method 942.05 in a 27 furnace at 550˚C. NDF and ADF using the fibretec System, AOAC methods 2002.04, and 973.18 (AOAC, 1990). In vitro DOMD and ME were determined using methods outlined by Roughan and Holland (1977).

3.3 Fourier transform infrared spectroscopy analysis

Infrared spectra of each sample were recorded using a ThermoFisher Scientific iS5 FTIR spectrometer with the attenuated total reflectance (ATR) iD7 (diamond crystal) attachment in reflectance mode, and OMNIC^{TM} software. The wavenumber range was recorded between 400 and 4000cm^{-1}, with 64 scans per spectrum with a spectral resolution of 4 cm^{-1} for each sample. The acquisition time for a single spectrum was 70 seconds. Background spectra were recorded prior to and during sample analysis with no sample present on the crystal whilst maintaining experiment conditions. The background spectra were checked periodically by running a blank sample, and a fresh background was recorded for data collection sessions longer than forty minutes. Beam alignment was checked prior to collection. For every sample five spectra were collected. The spectra were exported as a CSV excel file containing all of the data points. During analysis using python, an average was evaluated to further account for inhomogeneity.

3.4 Near infrared spectroscopy analysis

NIR spectral data of freeze-dried samples were captured by an ASD FieldSpec 4 High-Resolution spectrometer, using a high intensity handheld contact probe with a sampling diameter of 10mm (Kereszturi et al., 2018). The spectral range recorded reflectance between 350-2500 nm, with a spectral interval of 1nm. For each sample 5 spot measurements were taken, of which 10 spectra were recorded per spot, resulting in a total of 50 spectra recordings per sample. Before the first and between every sample recording, a spectralon white reference was used to calibrate the spectroradiometer contact probe and optimise the reflectance values. Once all the spectra were recorded, the files were analysed using View Spec Pro software where the spectra are averaged, leaving one mean spectra per sample. This single .txt file could then be imported into python for analysis.

3.5 Python for regression and model analysis

The data processing and analysis was mostly carried out using python software. There were four main steps to produce FTIR and NIR predictions and validation of these models. These include pre-processing, model building, prediction and evaluation of the model.

The pre-processing step as outlined in Table 4 manages all of the raw spectral data and wet chemical results. Although this step does not produce any results itself, it is critical data management to ensure all FTIR, NIR and wet chemistry file names match up correctly. Once verifying all files matched up, the raw spectra were plotted shown in figure 7. In this research, a savitzky golay (SG) pre-treatment was used, allowing the use of the whole spectra by reducing the strong water OH stretching band (Cleland et al, 2018). As a result, the effects of the water content are minimised providing a better regression model. This can be seen in figure 7.

Table 4: Basic steps to build and evaluate a regression model for analyte prediction using python.

1. Pre-Processing Read -> Nut lab data (nutritional analytical data) =>Select subset if required (tissue, species) Read -> Spectral files (ATR-FTIR and NIR) -> Check for missing data -> Output list of samples Read -> Spectral data (ATR-FTIR and NIR) Filtering -> Use Savitzky Golay filtering and check backgrounds using a gridplot	**2. Model Building** → Match analytical and spectral data for each sample using a dictionary. → Select analyte and spectrum type to predict, conduct preliminary PCA. → Scree, scores and loading plots → PLS Calibration (all data) ⇢ R^2(calib), MSE(calib) ⇢ R^2(CV), MSE(CV) → Outlier removal → Re-calibrate ⇢ R^2(calib), MSE(calib) ⇢ R^2(CV), MSE(CV) → Export model
3. Prediction → Load data → Load model → Train set ⇢ R^2(calib), MSE(calib) ⇢ R^2(CV), MSE(CV) → Test set ⇢ R^2(pre), MSE(pre) → Prediction for all samples	**4. Evaluation of model prediction** → RPD → RMSECV → R^2 vs n components to check bias vs overfitting

Once verifying all files matched up, the raw spectra were plotted shown in figure 7. In this research, a savitzky golay (SG) pre-treatment was used, allowing use of the whole spectra by reducing the strong water OH stretching band. As a result, the effects of the water content are minimised providing a better regression model. The result of this can be seen in figure 7 for both ATR-FTIR and NIR raw and SG treated spectra.

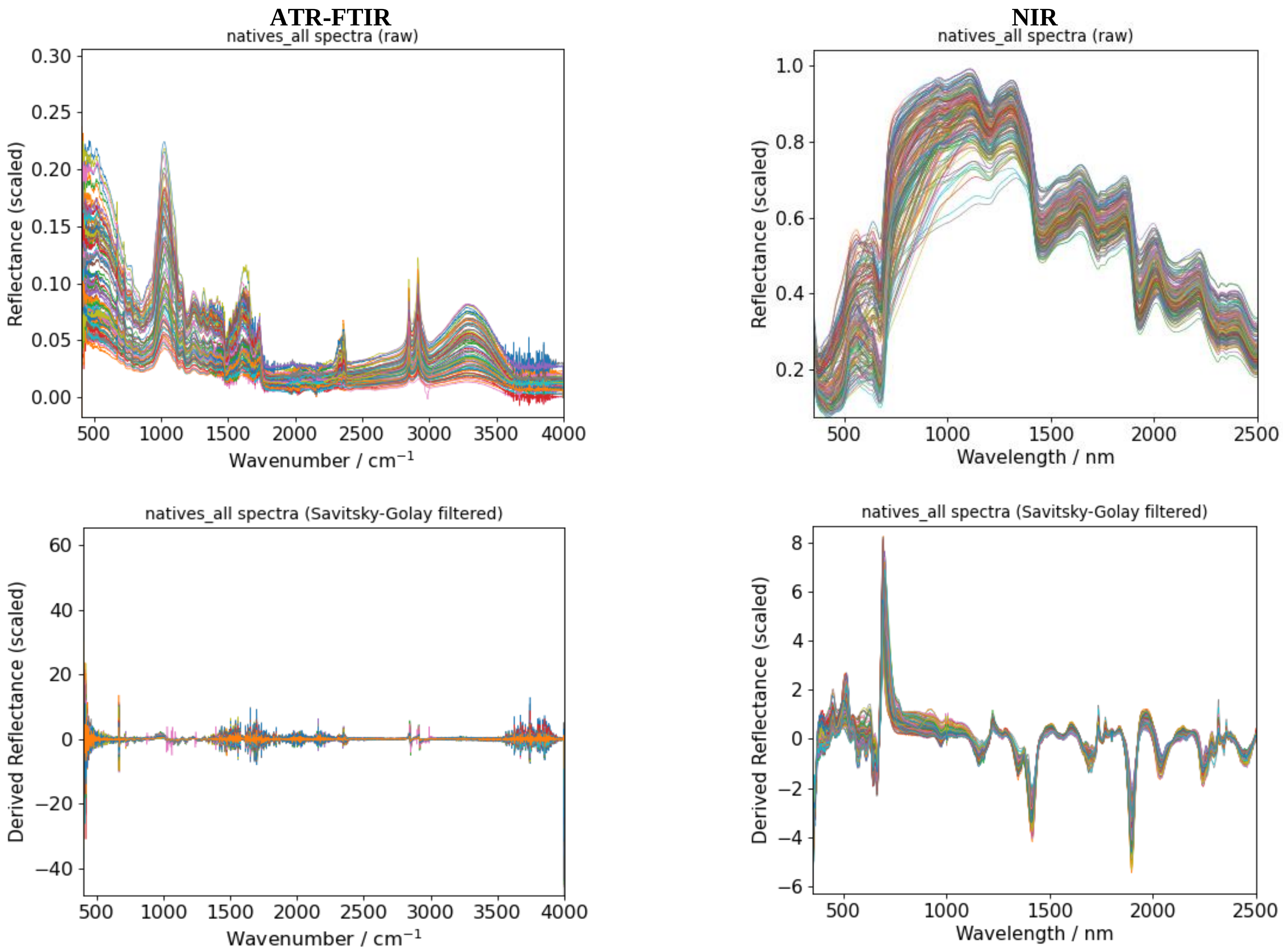


Figure 7: Spectra of ATR-FTIR (left) and NIR (right) showing the difference between raw spectra and Savitsky-Golay filtered spectra.

The second step, model building, first forms a dictionary for multivariate analysis. Principle component analysis (PCA) is used to dimensionally reduce the correlations within the spectra by transforming the large dimensionality to a smaller space, reducing the correlated components significantly. A scree plot was used to determine how many principal components to pick, usually 4-6. Using the scores and loading results shown in figure 8, a partial least squares regression (PLSR) is applied producing the first model with R^2(calibration (calib) and cross validation (CV)) and mean squared error (MSE) (calib and CV) using the secondary wet-chem data, maximising covariance (Shenk & Westerhaus, 1991; Lee et al., 2017). The model's calibration performance was evaluated using both cross validation and external validation datasets.

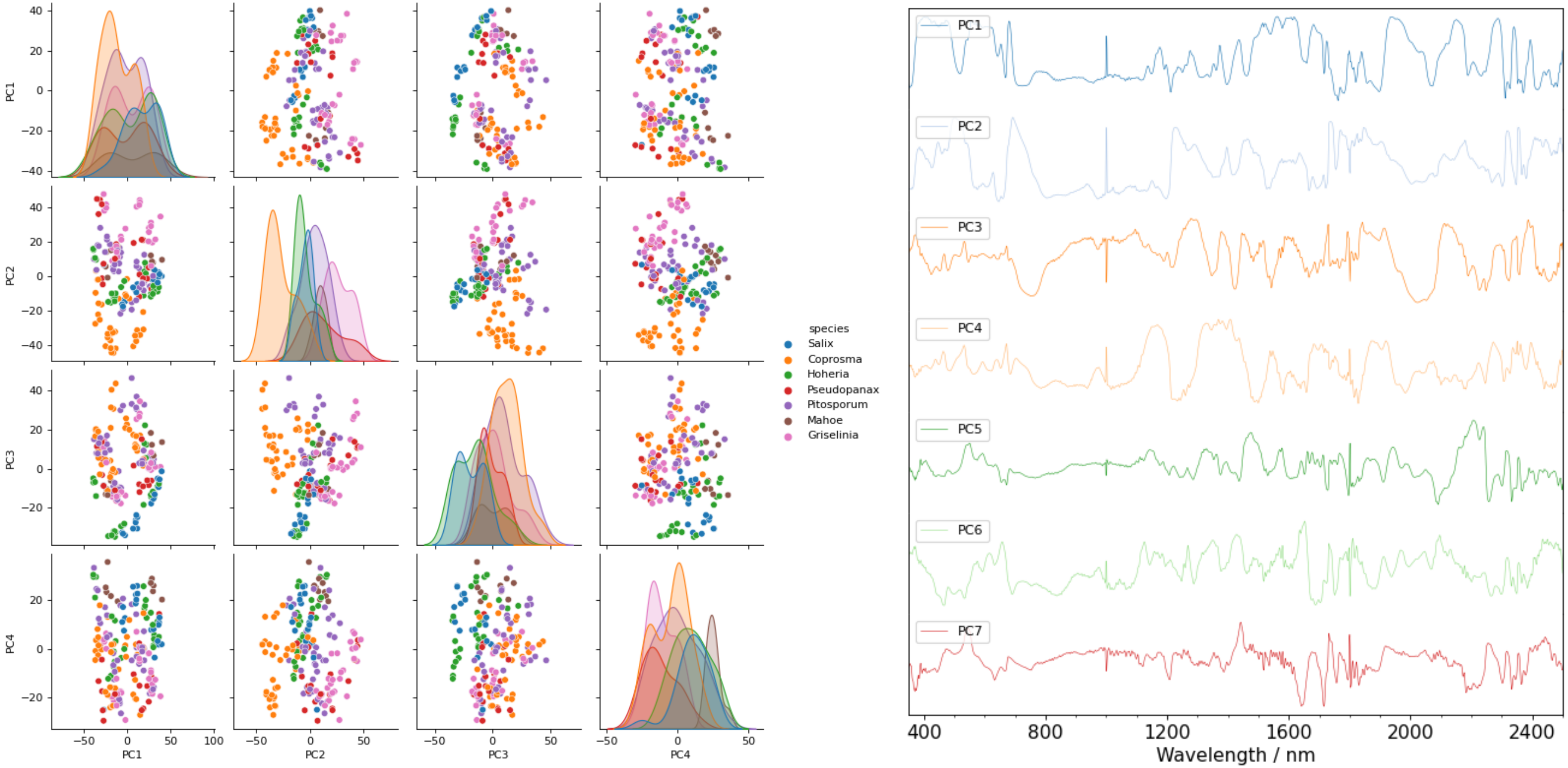


Figure 8: NIR scores scatter plot matrix (left) and loadings plot (right) for all samples.

Multivariate analysis can have two error weaknesses which can affect the model greatly, coming from large scores value and large residual values. These are called score outliers and residual outliers. To detect these outliers, Hotelling's T^2 and Q residuals must be calculated.

The Q residual essentially calculates how well the fit of a model is to i^{th} sample in x after projection, whereas the hotelling's t squared measures variation within the samples by setting a centre and confidence interval. The values outside the confidence interval are considered outliers. Plotting Q residuals vs hotelling's T square, often called an influence plot, displays both sets of outliers on one plot, with large scores outliers moving far right of the x axis and residual outliers high on the y axis (Lörchner et al, 2022; Hotelling, 1992; Joe, 2003). An example is given showing a samples/scores plot in figure 9. The blue/pink lines are 95% confidence intervals.

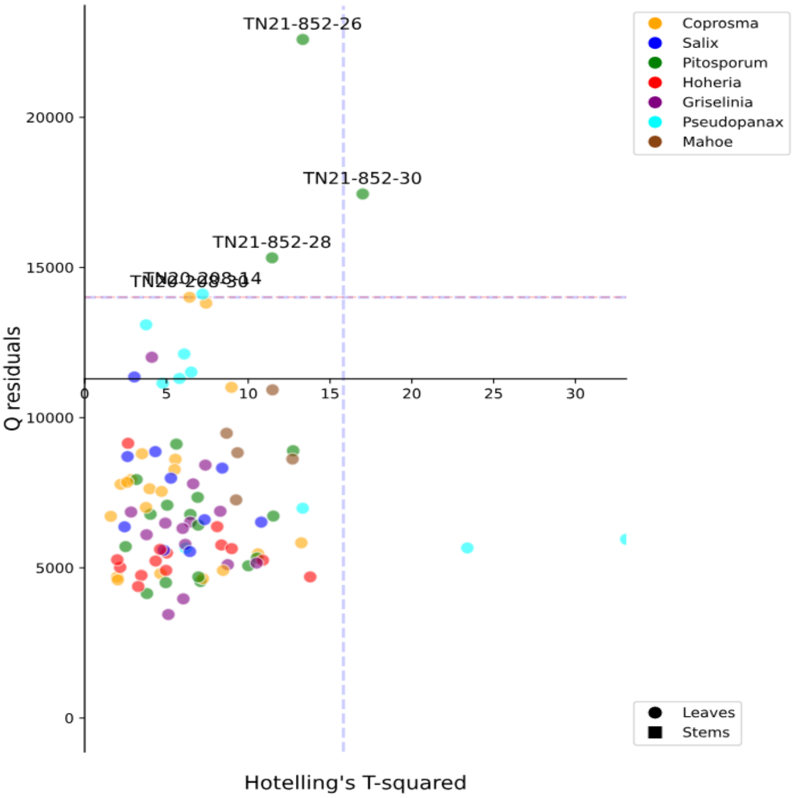


Figure 9: Plot of Q residuals vs Hotelling's T-squared for ATR-FTIR all leaves group, showing the outlier samples to be removed.

The mahalanobis distance and a threshold distance based on a specified confidence interval were calculated, filtering out any sample which is above the threshold intensity (Kniese & Schmidt, 2021; Leys et al, 2018).

The hotelling's t squared and q residual can be formulated from a model which describes loadings (P) and scores (T). The equation is given below where X is a matrix of m rows and n columns.

$$E = X - TP^T$$

The Q residual equation is given below, where e_i is the ith row of E, P_k is the matrix of the k loadings vectors retained in the model, I is the identity matrix of appropriate size, x_i is the i^{th} sample in X.

$$Q_i = e_i^T e = X_i^T (I - P_k P_k^T)_{x_i}$$

The hotelling's t square is made up of T^2 contributions given in the following equation.

$$T_i^2 = t_{con,i} t_{con,i}^T$$

$$t_{con,i} = t_i \lambda^{-1/2} P_k^T = x_i P_k \lambda^{-1/2} P_k^T$$

Where $t_{con,i}$ is $T_i^2$for ith sample 1 by k vector, $\lambda = T_k^T T_k/(m-1)$, P_k is the matrix of the k loadings.

$$D_M(\vec{x}_i) = \sqrt{(\vec{x}_i - \vec{u}_i)^T S^{-1} (\vec{x}_i - \vec{u}_i)}$$

Mahalanobis distance equation. Where $\vec{x}_i$ is a vector of values of the N dimensions of the ith in data point, $\vec{u}_i$ is a vector of the mean of each of the N dimensions (or columns) of interest, S^{-1} is the inverse of the NxN covariance matrix for all the data points.

Using an example (figure 9), the outliers for ATR-FTIR all leaves making up 92 samples was reduced to 87, removing sample ID: 29 (TN20-208-14), 45 (TN20-208-30), 137 (TN21-852-26), 139 (TN21-852-28) and 141 (TN21-852-30). Now the PLS could be run again to re-calibrate the model producing Outlier removed results. This regression model was then exported to be used for the prediction train set.

Prediction starts off by loading data with identical pre-treatment and outlier removal as the data used to create the model. This data is then split using a train-test-split tool, randomly assigning 75% to the train data set and 25% to the test data set. The model is loaded up and PLS applied, producing the results for training data and a model to be used for the test set. Using this freshly calibrated model from the training set, the model is applied to the test set, producing the R^2 (pred) and MSE (pred) results seen in tables 11-16. Prediction for all samples (excluding outliers) was also conducted due to low sample number per species and tissue type in the test set. As little as 2 samples for a given species and tissue type could end up in the random test set, so instead the whole set (excluding outliers) was used for the nutritional value prediction and post prediction evaluation statistics. Figure 10 shows how the data was split and used. The predicted values were exported to individual analyte csv files, with sample ID, observed (wet chem value) and predicted values in columns. These values were then averaged in accordance with species and tissue type and make up table 5-10 in the results.

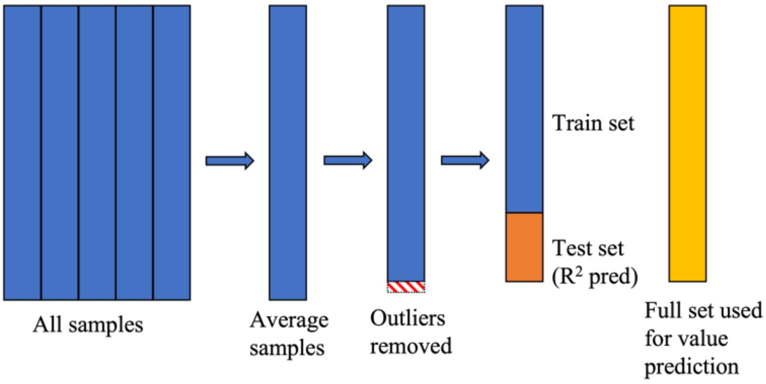


Figure 10: Flow chart showing how the samples are grouped for analysis in python.

3.6 Statistics

Root mean square error of cross validation (RMSECV) was used to select the optimum number of components for the regression model. The equation shows the calculation below:

$$RMSECV = \sqrt{\frac{\sum(y_{est} - y_{known})^2}{n}}$$

Evaluation of the model prediction was achieved using relative performance deviation (RPD) and root mean square error cross validation (RMSECV). Standard error of prediction (SEP) was used for the RPD calculation. The equations used to calculate these are given as:

$$Bias = \frac{\sum(O - P)}{n}$$

$$SEP = \sqrt{\frac{\sum(y_{est} - y_{known} - bias)^2}{n - 1}}$$

$$RPD = \frac{\sigma}{SEP}$$

Where σ is standard deviation of the observed value and n is the number of samples.

3.6.1 Post Prediction Statistics

As mentioned above, due to small sample numbers and having a test group of 25% some prediction models resulted in low R^2 and high MSE. To get around this shortcoming, the RPD, RMSECV, Bias, P-value paired t test, mean prediction error (MPE), relative prediction error (RPE) and concordance correlation coefficient (CCC) were calculated using the prediction output results. The code was made to output a CSV file list of predicted and observed values per each analyte, for all 171 samples.

Paired T test = $\frac{\sum(observed - predicted)}{\sigma \times n^{-0.5}}$

P test is done by calculating the probability using a two tailed t distribution.

Calculation of RPE:

$$MPSE = \frac{\sum(O-P)^2}{n}$$

$$MPE = \sqrt{MPSE}$$

$$RPE = \frac{MPE}{O}$$

Calculation of concordance correlation coefficient (CCC):

$$CCC = \frac{2 \times COV}{(SD_O^2 + SD_P^2) + (\mu_P - \mu_O)^2}$$

CCC value	Interpretation
0.21-0.4	Reasonable
0.41-0.6	Moderate
0.61-0.8	Significant
0.81-1	Almost perfect

Where COV is covariance, SD is standard deviation and μ is mean.

CCC is useful as it does not only factors in random error but also systematic bias whilst comparing two groups.

4.0 Results

4.1 Predicted values

The results for this section are split up into separate analytes sections starting with NDF and ending with ADF, found in tables 5-10. For all analyte results there was a difference between the stems and leaves values. The following will identify the highest and lowest values regarding species and tissue type. In addition, FTIR and NIR values are compared. This section includes histograms (figure 11-16), to show the distribution of all of the samples grouped by species for each analyte, to more clearly visualise the similarities and differences between species, where count is number of samples within the analyte range.

The letters A, S and L will be used to indicate which tissue group a result value is part of, all, stems or leaves.

4.1.1 NDF

Starting with NDF %, there was a clear trend that leaf samples are lower than stem samples. The lowest FTIR result comes from Pseudopanax with 29.33 A, 35.21 S and 20.64 L. These FTIR results were similarly mirrored by NIR results also placing Pseudopanax as the lowest species with 30.89 A, 34.82 S and 20.41L.

The next highest species was Griselinia, with 37.34 (FTIR) and 37.59 (NIR) all, 32.01 (FTIR) and 31.05 (NIR) leaves, and 47.14 (FTIR) and 48.14 (NIR) stems. Interestingly, Coprosma which has a higher mean than Griselinia for all 41.42(FTIR), 39.29(NIR) and leaves 34.81(FTIR) 35.02(NIR), had a lower mean than Griselinia in stems 45.13(FTIR) and 44.47(NIR). This was not a coincidence as the same pattern was seen in the wet chemistry.

Coprosma, Hoheria and Pittosporum had similar values in all 41.42-42.45 (FTIR) and leaves 39.27-43.67 (NIR). As mentioned above, Coprosma has a low value for stems, so it was not similar to Hoheria 50.92 (FTIR) and 50.21 (NIR), or Pittosporum 49.63 (FTIR) 49.78 (NIR) for this tissue however, Salix is 48.92 (FTIR) and 50.68 (NIR).

Salix was the second highest species with values 47.25 (FTIR) 47.20 (NIR) all and 42.13 (FTIR) and 43.14 (NIR) leaves. This leaves Mahoe as the highest species with values of 56.26 (FTIR) and 55.74 (NIR) all which was noticeably higher than Salix. The values between Salix and Mahoe 44.06 (FTIR) and 44.82 (NIR) are similar for leaves. The largest difference was in the stems where Mahoe has a clear difference in value, 61.83 (FTIR) and 66.36 (NIR) compared to the other species. Something worth noting for stems was that the difference between FTIR and NIR in Mahoe was large with the NIR and wet chemistry being very similar (66.36) compared with 61.83 (FTIR).

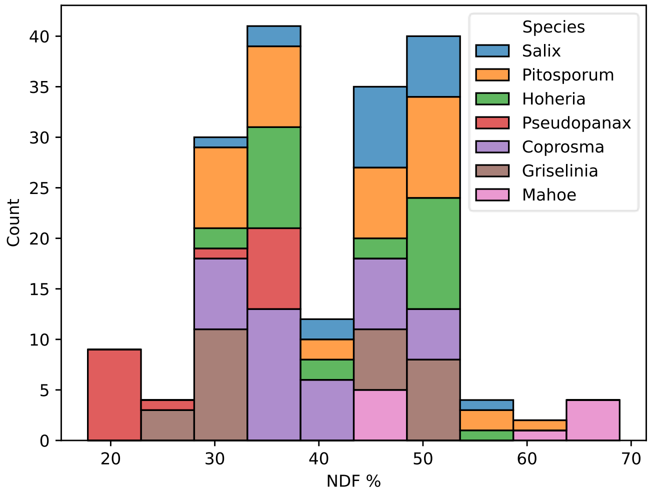


Figure 11: Histogram showing the distribution of NDF% content for each species.

4.1.2 ME

Unlike NDF, ME produces higher values in leaves than stems. There are only small differences between FTIR, NIR and wet chemistry values across the species and tissue type. Interestingly, Mahoe which produced the highest NDF value had the lowest ME value. Mahoe results were clearly lower for all 8.01MJ/kg (FTIR), 8.10MJ/kg (NIR), leaves 9.38MJ/kg (FTIR), 9.44MJ/kg (NIR) and Stems 6.92MJ/kg (FTIR) 6.82MJ/kg (NIR). Hoheria was the next lowest values with 9.85MJ/kg (FTIR) 9.82MJ/kg (NIR) all and 9.04MJ/kg (FTIR) 9.00MJ/kg (NIR) stems. Not far off from these values was Pittosporum all, 10.18MJ/kg (FTIR) 9.92MJ/kg (NIR) and stems 9.29MJ/kg (FTIR) 9.07MJ/kg (NIR). The NIR values are more similar between species. As for leaves, Hoheria 10.74MJ/kg (FTIR) 10.53MJ/kg (NIR) was very similar to Salix 10.56MJ/kg (FTIR) 10.67MJ/kg (NIR). Coprosma and Griselinia were very similar for all tissue types and reported values greater than the previous species in all and leaves. Salix 9.86MJ/kg (FTIR) 9.73MJ/kg (NIR) has higher ME in the stems than both coprosma 9.59MJ/kg (FTIR) 9.7MJ/kg (NIR) and Griselinia 9.59MJ/kg (FTIR) 9.65MJ/kg (NIR). This leaves Pseudopanax reporting the highest ME of all the species with 11.25 (FTIR) 11.04 (NIR) all, 11.97 (FTIR) 11.81 (NIR) leaves and 10.36 (FTIR) 10.34 (NIR) stems.

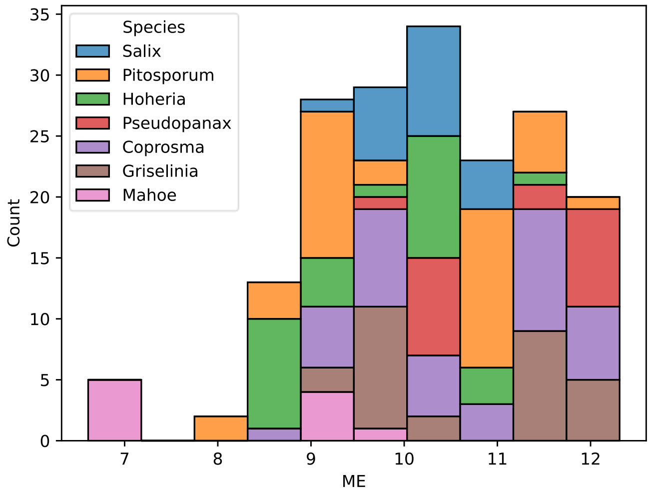


Figure 12: Histogram showing the distribution of ME% content for each species.

4.1.3 CP

The CP results are similar between FTIR, NIR and wet chemistry across all tissue types. Crude protein has the highest values in leaves compared to stems and all, with the highest species for all tissue types in Hoheria.

The order of the lowest three species stays the same across all three tissue types, with the lowest Pseudopanax 4.89% A, 5.79% L and 3.13% S for FTIR and 4.69% A, 5.33% L and 2.88% S for NIR. The second lowest was Griselinia 5.27% A, 6.33% L and 3.59% S for FTIR and 4.95% A, 6.08% L and 3.71% S for NIR. The third lowest was Pittosporum 6.01% A, 7.54% L and 4.59% S for FTIR, and 5.82% A, 7.33% L and 4.51% S for NIR.

Salix was the second highest species in all (7.84% FTIR and 9.09% NIR), and leaves (11.23% FTIR and 11.20% NIR) but dropped to the fourth highest species for stems (4.80% FTIR and 5.13% NIR). Mahoe was similar to Salix for all but third in leaves and second highest in stems (6.47% FTIR and 6.04% NIR). Coprosma was the fourth highest species sitting above Pittosporum and below Mahoe. Coprosma moves above Salix to third highest in stems (5.41% FTIR and 5.24% NIR). The most significant difference from stems to leaf values occurred in Hoheria and Salix, jumping from 6.82% FTIR and 7.78% NIR (Hoheria) and 4.80% FTIR and 5.23% NIR (Salix) stems, to 11.71% FTIR, 12.56% NIR (Hoheria) and 11.23% FTIR, 11.20% NIR (Salix) for leaves.

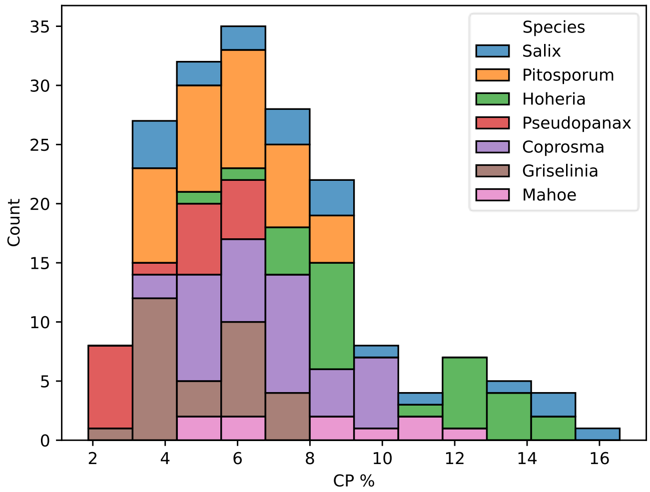


Figure 13: Histogram showing the distribution of CP% content for each species.

4.1.4 *In Vitro* DOMD

The results for DOMD are very similar to ME because of their relationship being ME=0.16xDOMD. Therefore, the trends seen in ME may also be seen in DOMD. As expected, Pseudopanax had the highest value across all species with 70.41% (FTIR) 68.91% (NIR) all, 74.00% (FTIR) 74.30% (NIR) leaves and 64.49% (FTIR) 64.94% (NIR) for stems. Mahoe has the lowest values with 50.50% (FTIR) 50.91% (NIR) all, 57.77% (FTIR) 58.48% (NIR) leaves and 43.73% (FTIR) 42.58% (NIR) for stems.

There were two pairs of similar values with the addition of Salix which fits differently to both groups. Hoheria and Pittosporum have very similar results 61.20% (FTIR) 60.53% (NIR) and 62.80% (FTIR) 61.67% (NIR) all, 67.99% (FTIR) 65.83% (NIR) and 68.57% (FTIR) 69.97% (NIR) leaves and 56.17% (FTIR) 55.90% (NIR) and 57.16% (FTIR) 57.56% (NIR) stems respectively. Salix has a slightly higher value for all 63.46% (FTIR) 64.70% (NIR) and stems 65.80% (FTIR) 66.13% (NIR), but when it comes to leaves, Salix switches to having slightly lower values than Hoheria and Pittosporum.

Coprosma, 65.4% (FTIR) 66.67% (NIR) all, 71.30% (FTIR) 71.73% (NIR) leaves and 60.57% (FTIR) 60.26% (NIR) stems, and Griselinia, 66.09% (FTIR) 66.66% (NIR) all 71.23% (FTIR) 72.56% (NIR) leaves and 60.65% (FTIR) 60.13% (NIR) stems, are also very similar to each other and have higher values than the previous species. Salix was very similar to this pair for stem values. None of the values in FTIR or NIR are significantly different from the wet chem values. The values had no pattern of under or over predicting from the wet chemistry.

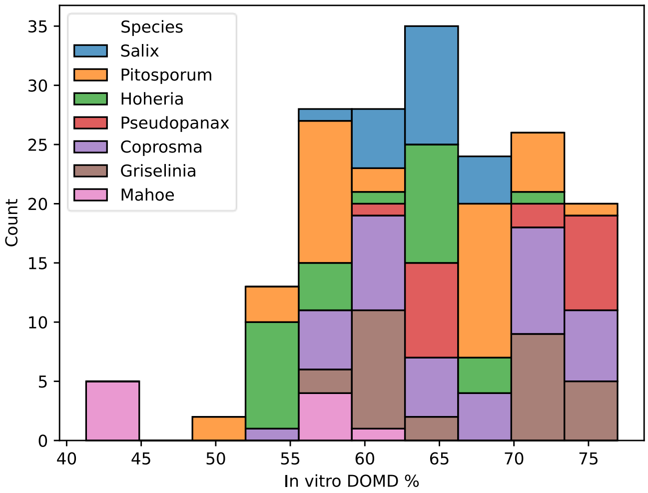


Figure 14: Histogram showing the distribution of In Vitro DOMD% content for each species.

4.1.5 Ash

The tissue trend for Ash % was that leaf values are greater than stems with the exception of Pittosporum and Pseudopanax, which have a higher stem value. The order of highest to lowest species was similar for all and leaves but deviated in the stems results.

Mahoe, 10.58% (FTIR) 10.00% (NIR) all, 12.76% (FTIR) 11.78% (NIR) leaves and 8.68% (FTIR) 7.91% (NIR) stems and Hoheria, 9.90% (FTIR) 10.67% (NIR) all, 11.69% (FTIR) 12.16% (NIR) leaves and 8.76% (FTIR) 9.40% (NIR) stems, had the highest values for all, leaves and stems. These two species swap highest depending on which prediction method, FTIR or NIR was used. For example, in all, Mahoe is greater than Hoheria using FTIR, but Hoheria was greater when using NIR. This also occurred in leaves where again Mahoe was larger than Hoheria for FTIR, whilst Hoheria was larger than Mahoe using NIR. The wet chemistry values show Mahoe was greater than Hoheria and the prediction for NIR or Mahoe was 0.95 lower than the wet chem compared to FTIR, just 0.03 different. This was seen again in stems for Mahoe with FTIR 8.68%, NIR 7.91% compared with the wet chem 8.88%. So, there was a 0.2 difference with FTIR and 0.77 for NIR.

The next largest value was Pittosporum with 7.75% (FTIR) 7.86% (NIR) all, 7.85% (FTIR) 7.13% (NIR) leaves and 8.01% (FTIR) 7.88% (NIR) stems. As mentioned above the stem and leaf values vary from the other species. A slightly lower species was Coprosma, which was similar to Pseudopanax for all and leaves, but Pseudopanax was greater than Coprosma for stems.

Griselinia was the second lowest species for all 6.46% (FTIR) 5.93% (NIR) and stems 5.35% (FTIR) 5.56% (NIR) however, has a higher NIR for Pseudopanax and FTIR value than Coprosma and Pseudopanax for leaves. The lowest species by far was Salix with values of 4.85% (FTIR) 4.79% (NIR) all, 5.43% (FTIR) 5.40% (NIR) leaves and 4.37% (FTIR) 3.94% (NIR) stems. Interestingly, the prediction values for FTIR and NIR were all slightly higher than the wet chemistry values.

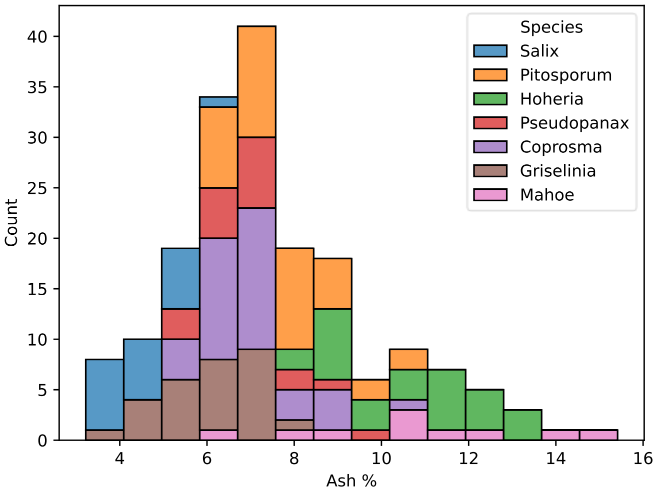


Figure 15: Histogram showing the distribution of ASH% content for each species.

4.1.6 ADF

The general trend for ADF was that stem values were higher than leaves. Mahoe has the highest predicted value for ADF with 41.83% (FTIR) 43.00% (NIR) all and 55.47% (FTIR) 57.01% (NIR) stems. The leaves 29.95% (FTIR) 29.32% (NIR) shared similar values to Salix 29.61% (FTIR) and 29.53% (NIR). Salix was the second highest in all 33.97% (FTIR) 34.64% (NIR). The next lowest values were similar between Coprosma, Griselinia, Hoheria and Pittosporum ranging from 28.38-38.76% (FTIR) and 28.16-31.94% (NIR).

Salix was similar to Griselinia and Pittosporum for stems ranging from 37.44-38.76% (FTIR) and 38.09-40.43% (NIR).

Coprosma placed interestingly being the third highest value for leaves 23.72% (FTIR) 24.30% (NIR) but then the second lowest for stems 35.80% (FTIR) 34.99% (NIR). Psudopanax was the lowest species across all tissue types 21.78% (FTIR) 21.77% (NIR) all, 16.10% (FTIR) 15.77% (NIR) leaves and 31.79% (FTIR) 26.52% (NIR) for stems. The Pseudopanax FTIR prediction of 31.79% compared with NIR 26.52% and 26.86% wet chemistry is very off. This was the only result with such a large margin, so there has potentially been some error in the prediction or calibration.

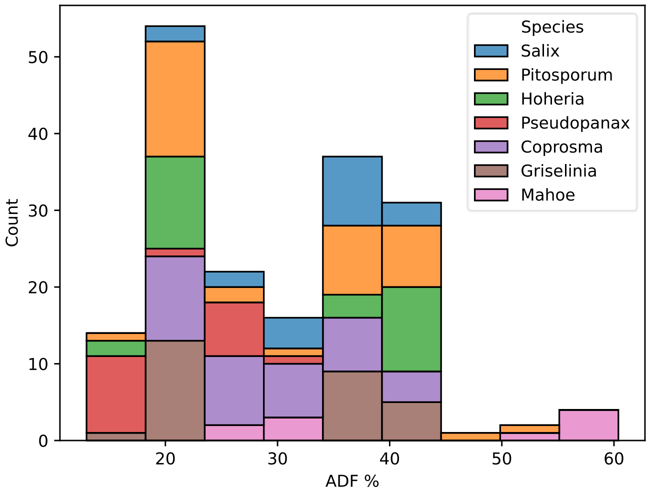


Figure 16: Histogram showing the distribution of ADF% content for each species.

Table 5: Results displaying the mean value predicted for NDF % using FTIR and NIR, with the actual wet chem value recorded.

NDF %	FTIR	NIR	Wet Chem
All			
Coprosma	41.42	39.29	39.70
Griselinia	37.34	37.59	39.42
Hoheria	42.45	43.67	43.20
Mahoe	56.26	55.74	56.02
Pittosporum	41.67	41.82	42.31
Pseudopanax	29.33	30.89	27.17
Salix	47.25	47.20	46.25
Leaves			
Coprosma	34.81	35.02	35.17
Griselinia	32.01	31.05	30.35
Hoheria	36.15	36.94	35.42
Mahoe	44.06	44.82	45.69
Pittosporum	34.84	33.33	35.11
Pseudopanax	20.64	20.41	20.80
Salix	42.13	43.14	42.58
Stems			
Coprosma	45.13	44.47	44.73
Griselinia	47.14	48.14	48.50
Hoheria	50.92	50.21	50.98
Mahoe	61.83	66.36	66.36
Pittosporum	49.63	49.78	49.50
Pseudopanax	35.21	34.82	34.26
Salix	48.92	50.68	49.93

Table 6: Results displaying the mean value predicted for ME % using FTIR and NIR, with the actual wet chem value recorded.

ME MJ/kg	FTIR	NIR	Wet Chem
All			
Coprosma	10.58	10.62	10.60
Griselinia	10.57	10.66	10.64
Hoheria	9.85	9.82	9.75
Mahoe	8.01	8.10	8.07
Pittosporum	10.18	9.92	10.08
Pseudopanax	11.25	11.04	11.20
Salix	10.14	10.36	10.19
Leaves			
Coprosma	11.43	11.42	11.40
Griselinia	11.57	11.71	11.65
Hoheria	10.74	10.53	10.62
Mahoe	9.38	9.44	9.32
Pittosporum	10.97	11.11	10.99
Pseudopanax	11.97	11.81	11.96
Salix	10.56	10.67	10.53
Stems			
Coprosma	9.59	9.70	9.71
Griselinia	9.59	9.65	9.63
Hoheria	9.04	9.00	8.88
Mahoe	6.92	6.82	6.83
Pittosporum	9.29	9.07	9.18
Pseudopanax	10.36	10.34	10.36
Salix	9.86	9.73	9.85

Table 7: Results displaying the mean value predicted for DOMD % using FTIR and NIR, with the actual wet chem value recorded.

DOMD %	FTIR	NIR	Wet Chem
All			
Coprosma	65.40	66.67	66.25
Griselinia	66.09	66.66	66.51
Hoheria	61.20	60.53	60.92
Mahoe	50.50	50.91	50.46
Pittosporum	62.80	61.67	63.00
Pseudopanax	70.41	68.91	70.00
Salix	63.46	64.70	63.69
Leaves			
Coprosma	71.30	71.73	71.24
Griselinia	71.23	72.56	72.81
Hoheria	67.99	65.83	66.35
Mahoe	57.77	58.48	58.23
Pittosporum	68.57	69.97	68.68
Pseudopanax	74.00	74.30	74.75
Salix	65.80	66.13	65.82
Stems			
Coprosma	60.57	60.26	60.70
Griselinia	60.65	60.13	60.21
Hoheria	56.17	55.90	55.50
Mahoe	43.73	42.58	42.70
Pittosporum	57.16	57.56	57.32
Pseudopanax	64.49	64.94	64.73
Salix	60.19	61.57	61.57

Table 8: Results displaying the mean value predicted for CP % using FTIR and NIR, with the actual wet chem value recorded.

CP %	FTIR	NIR	Wet Chem
All			
Coprosma	6.84	6.66	6.71
Griselinia	5.27	4.95	4.97
Hoheria	9.55	10.02	10.18
Mahoe	7.98	8.04	8.15
Pittosporum	6.01	5.82	5.72
Pseudopanax	4.86	4.69	4.16
Salix	7.84	8.09	8.10
Leaves			
Coprosma	8.27	8.05	8.05
Griselinia	6.33	6.08	6.31
Hoheria	11.71	12.56	12.52
Mahoe	9.81	10.03	10.20
Pittosporum	7.54	7.33	6.94
Pseudopanax	5.76	5.33	5.35
Salix	11.23	11.20	11.09
Stems			
Coprosma	5.41	5.24	5.23
Griselinia	3.59	3.71	3.63
Hoheria	6.82	7.78	7.83
Mahoe	6.47	6.04	6.09
Pittosporum	4.59	4.51	4.51
Pseudopanax	3.13	2.88	2.83
Salix	4.80	5.13	5.10

Table 9: Results displaying the mean value predicted for Ash % using FTIR and NIR, with the actual wet chem value recorded.

Ash %	FTIR	NIR	Wet Chem
All			
Coprosma	7.19	6.96	7.01
Griselinia	6.46	5.93	6.10
Hoheria	9.90	10.67	10.60
Mahoe	10.58	10.00	10.80
Pittosporum	7.75	7.86	7.74
Pseudopanax	6.82	6.94	7.04
Salix	4.85	4.79	4.56
Leaves			
Coprosma	7.16	7.49	7.35
Griselinia	7.25	6.64	6.88
Hoheria	11.69	12.16	11.96
Mahoe	12.76	11.78	12.73
Pittosporum	7.85	7.13	7.66
Pseudopanax	6.71	6.77	6.42
Salix	5.43	5.40	5.26
Stems			
Coprosma	6.86	6.81	6.62
Griselinia	5.35	5.56	5.33
Hoheria	8.76	9.40	9.24
Mahoe	8.68	7.91	8.88
Pittosporum	8.01	7.88	7.82
Pseudopanax	7.42	7.64	7.72
Salix	4.37	3.94	3.85

Table 10: Results displaying the mean value predicted for ADF % using FTIR and NIR, with the actual wet chem value recorded.

ADF %	FTIR	NIR	Wet Chem
All			
Coprosma	29.10	29.79	29.61
Griselinia	28.38	29.18	29.76
Hoheria	31.05	28.16	29.85
Mahoe	41.83	43.00	43.51
Pittosporum	30.43	31.94	30.53
Pseudopanax	21.78	21.77	20.81
Salix	33.97	34.64	33.92
Leaves			
Coprosma	23.72	24.20	24.35
Griselinia	20.89	21.22	20.89
Hoheria	19.89	19.58	19.37
Mahoe	29.95	29.31	30.10
Pittosporum	21.89	21.01	22.37
Pseudopanax	16.10	15.77	15.36
Salix	29.61	29.53	29.50
Stems			
Coprosma	35.80	34.99	35.45
Griselinia	38.34	38.09	38.62
Hoheria	40.61	40.44	40.33
Mahoe	55.47	57.01	56.93
Pittosporum	37.44	40.43	38.70
Pseudopanax	31.79	26.52	26.86
Salix	38.76	39.10	38.34

4.2 Regression Results

The regression results in this section, report how well the models were in terms of coefficient of determination R^2 and mean squared error (MSE) for calibration (calib) and cross validation (CV) sets. The test data set is the prediction set only reporting R^2 prediction and MSE prediction. The performance of the prediction was determined with relative performance deviation (RPD) and root mean square error cross validation (RMSECV).

There were too few samples to predict individual species accurately using this method, so these results use one model for all species. The individual R^2 for each species was determined with other results from a statistical analysis of the predicted values. These will be reported in the next results section.

To maintain the same interpretation the following results, value ranges were used to determine quality. An excellent R^2 is greater than 0.9, whilst a good R^2 is considered between 0.7-0.89, moderate prediction between 0.5-0.69 and poor $R^2<0.5$. R^2 CV is expected to be lower than the calibration values with R^2 CV 0.51-0.74 considered useful for quality prediction and greater considered excellent (Clingensmith et al, 2019). A lower MSE value suggests better model accuracy. RPD equal or greater than 2 is considered a high quality prediction with RPD>3 considered excellent (Clingensmith et al, 2019).

The letters A, S and L will be used to indicate which tissue group a result value is part of, all, stems or leaves.

4.2.1 ME

The results for ME are very promising with excellent R^2 calibration and cross validation, low MSE and high RPD.

All Data produced excellent R^2(calib) for both FTIR and NIR in all 0.96 and 0.92, stems 0.97 and 0.94, and leaves 0.92 and 0.95 respectively. Most R^2(calib) stayed the same or improved once outliers were removed with FTIR 0.96 A, 0.99 S, 0.94 L and NIR 0.92 A 0.93 S and 0.97 L. NIR stems was the only value to drop from 0.94 to 0.93 whilst FTIR stems increased from 0.97 to 0.99, the highest recorded.

Similarly, with the R^2(CV) all but two results across all data and outliers removed had excellent R^2CV>0.74. The two values below, leaves all data NIR 0.71 and leaves outliers removed FTIR 0.73 were just under the lower limit for excellent however, are still considered very useful for quality prediction. The highest R^2CV in All data was 0.86 A FTIR and 0.89 A NIR and outlier removed was 0.86 A FTIR and 0.89 L NIR.

The training data set also produced excellent R^2(calib) with very similar values 0.92 A, 0.96 S and 0.93 L for FTIR, and 0.91 A, 0.95 S and 0.94 L for NIR. R^2(CV) were mostly excellent with two values in the range of useful for quality prediction for FTIR 0.83 A, 0.62 S and 0.62 L, whereas NIR only had excellent values 0.86 A, 0.91 S and 0.81 L.

The Test Data set had lower R^2(pred) than all previous results with only one excellent and the other two good for NIR, 0.83 A, 0.85 S and 0.91 L, and good results across all tissue types for FTIR, 0.76 A, 0.79 S and 0.83 L.

The MSE(calib) across the all data set is good with the lowest in outliers removed, 0.05 A, 0.01 S and 0.04 L for FTIR and 0.11 A, 0.06 S and 0.01 L for NIR, with the highest in the test data set 0.29 A, 0.15 S and 0.09 L FTIR and 0.15 A, 0.17 S and 0.04 L NIR. The

MSE(CV) showed positive results with no large values observed. FTIR had lower MSE values than NIR for All and Stems except for test set where FTIR was higher.

RPD for ME predictions for both FTIR and NIR were excellent RPD>3. FTIR had 3.00 A, 3.52 S and 3.25 L, whilst NIR had 3.17 A, 3.17 S and 3.89 L. This suggests FTIR performed best for Stems and NIR best for Leaves. The RMSECV was low overall with the highest value being 0.88 and lowest 0.18. FTIR was similar to NIR for all and leaves tissue groups, but larger than NIR for stems with 0.40 A, 0.88 S and 0.23 L FTIR and 0.37 A, 0.28 S and 0.18 L for NIR.

Table 11:Coefficient of determinations R2, Mean Square Error (MSE) and Relative Performance Deviation (RPD) for Fournier Transformed Infrared (FTIR) and Near Infrared (NIR) Metabolisable Energy (ME) calibrations (calib) and cross-validation (CV) for stems, leaves and steams plus leaves (All) using all the data, the data after removing the outliers, a training data set (75% of data) and a test data set (25% of data).

		All		**Stems**		**Leaves**	
ME MJ/kg		**FTIR**	**NIR**	**FTIR**	**NIR**	**FTIR**	**NIR**
All Data	n	181	181	89	89	92	92
	R^2(calib)	0.95	0.92	0.97	0.94	0.92	0.95
	R^2 (CV)	0.86	0.89	0.80	0.88	0.77	0.71
	MSE(calib)	0.07	0.11	0.02	0.05	0.05	0.03
	MSE(CV)	0.20	0.16	0.15	0.09	0.13	0.16
Outliers Removed	n	171	171	84	84	87	88
	R^2(calib)	0.96	0.92	0.99	0.93	0.94	0.97
	R^2 (CV)	0.86	0.87	0.82	0.88	0.73	0.89
	MSE(calib)	0.05	0.11	0.01	0.06	0.04	0.01
	MSE(CV)	0.20	0.18	0.14	0.09	0.15	0.06
Training Data set	n	129	129	63	63	66	66
	R^2(calib)	0.92	0.91	0.96	0.95	0.93	0.94
	R^2 (CV)	0.83	0.86	0.62	0.91	0.62	0.81
	MSE(calib)	0.12	0.13	0.03	0.05	0.04	0.03
	MSE(CV)	0.26	0.21	0.29	0.08	0.21	0.10
Test Data set	n	42	42	21	21	21	21
	R^2(pred)	0.76	0.83	0.79	0.85	0.83	0.91
	MSE(pred)	0.29	0.15	0.15	0.17	0.09	0.04
	RPD	3.00	3.17	3.52	3.17	3.25	3.89
	RMSECV	0.40	0.37	0.88	0.28	0.23	0.18

4.2.2 NDF %

The results for NDF% show high R^2 until the test set data, dropping significantly for FTIR All and Stems. RPD is above 2 for all results and the RMSECV is low.

All data showed mixed excellent and good results for R^2(calib) in FTIR and NIR. FTIR had one excellent in all and two good values for stems and leaves, 0.92 A, 0.84 S and 0.85 L. NIR had one excellent value in stems and two good values in all and leaves, 0.89 A, 0.91 S and 0.86 L. The R^2(CV) results were also positive with two excellent values in NIR All 0.77 and stems 0.75. The rest of the results fell within the useful for quality prediction range. Three values on the lower end were FTIR stems 0.55 and FTIR and NIR leaves, 0.58 and 0.54 respectively.

Removing the outliers increased the R^2(calib) for FTIR all, stems and NIR leaves above 0.90, making them excellent. NIR stems had no change, whilst NIR all and FTIR leaves decreased resulting in 0.94 A, 0.98 S and 0.83 L for FTIR and 0.86 A, 0.91 S and 0.92 L for NIR. The R^2(CV) only had one noticeable change in NIR leaves increasing from 0.54 to 0.71. As it did not cross the threshold for excellent it remains a good value useful for quality prediction. The training data set once again showed very high R^2(calib) with 0.89 A, 0.90 S and 0.80 L for FTIR and 0.87 A, 0.92 S and 0.82 L for NIR. Both excellent R^2(calib) are in stems, with the all values very close to the threshold for excellence. Leaves showed good R^2(calib) and are similar between FTIR 0.80 and NIR 0.82. The R^2(CV) had a notable decrease for FTIR across all tissue types and NIR leaves. NIR all and stems values were above excellent. FTIR all and NIR leaves remained in the useful for quality prediction range. FTIR stems and leaves values dropped under the 0.51 limit suggesting relatively poor models, 0.67 A 0.24 S and 0.47 L for FTIR and 0.76 A, 0.75 S and 0.56 L for NIR.

The test data set took a massive drop for R^2(pred) for FTIR all and stems 0.56 and 0.51, moving them below good. The rest of the results take a slight decrease in R^2(pred) but are still considered good, 0.84 L FTIR, 0.70 A and 0.76 S for NIR. NIR stems 0.90 was considered excellent and the highest value for R^2(pred).

MSE(calib) for all data is similar across FTIR 8.24 A, 8.83 S and 8.04 L whilst NIR had a larger range 11.29 A, 5.08 S and 7.21 L. MSE(CV) is significantly higher with 27.66 A, 25.45 S and 22.08 L for FTIR, and 23.56 A, 13.99 S and 24.27 L for NIR.

Outliers removed has slightly lower MSE than all data with the exception of all NIR which increased from 11.29 A to 14.16 A. A very low MSE is in stems FTIR 1.00 which dropped from 8.83. MSE(CV) is similar to that in all data with 28.77 A, 26.33 S and 20.89 L for FTIR, and 23.64 A, 13.59 S and 14.81 L for NIR. The NIR leaves value 14.81 is the only one notably different dropping from 24.27 in all data. Training data set produces similar MSE to all data and outliers removed, with slight increase in all for FTIR 11.29 A, and NIR 12.99 A, as well as NIR leaves 9.59. The MSE(CV) increased for FTIR models with the largest in stems, 34.92 A, 47.72 S and 28.24 L. NIR models showed results very similar to those in all data 23.65 A, 14.24 S and 23.30 L. MSE(pred) for the test data were higher for FTIR and NIR all, 40.43 A and 22.68 A, as well as FTIR stems 16.41 S than in previous data sets. NIR stems and leaves, 6.15 S and 9.00 L, and FTIR leaves 8.88 L are similar to the training data. The RPD for all tissues and methods were above 2, producing good models. All and leaves results were similar between methods, 2.33 A and 2.28 L FTIR and 2.37 A and 2.31 L NIR. Stems had the highest RPD for FTIR and NIR with excellent RPD for NIR 3.4 S whilst FTIR was 2.54 S. RMSECV was relatively low and similar when comparing between methods for each tissue type, 4.34 and 4.25 A, 2.95 and 2.25 S and 3.32 and 3.09 L for FTIR and NIR respectively.

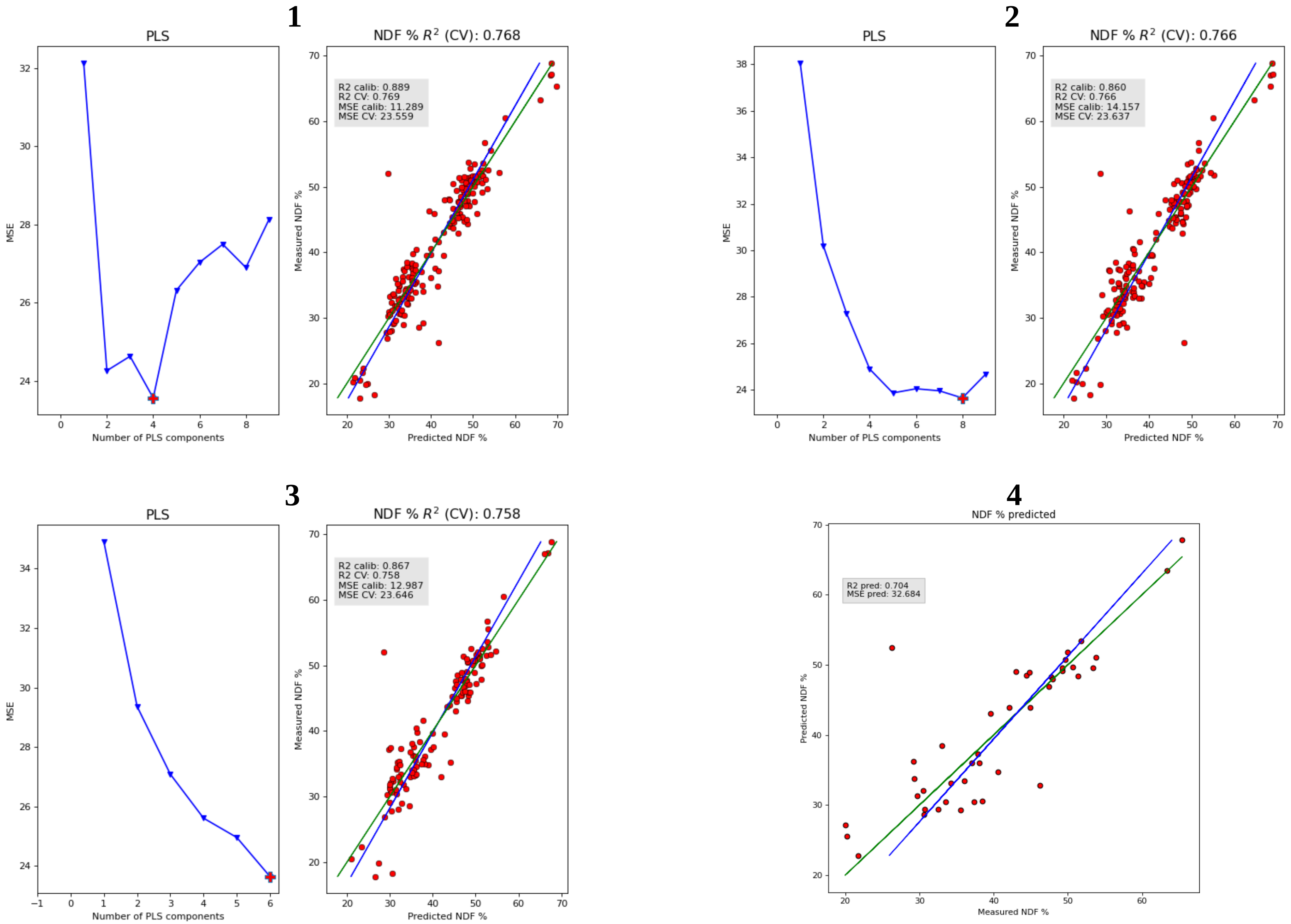


Figure 17: PLSR calibration and prediction results using NIR for NDF%. 1: All Data, 2: Outliers Removed, 3: Training Data Set, 4 Test Data Set.

Table 12:Coefficient of determinations R2, Mean Square Error (MSE) and Relative Performance Deviation (RPD) for Fournier Transformed Infrared (FTIR) and Near Infrared (NIR) Neutral Detergent Fiber (NDF %) calibrations (calib) and cross-validation (CV) for stem, leaves and stems plus leaves (All) using all the data, the data after removing the outliers, a training data set (75% of data) and a test data set (25% of data).

		All		**Stems**		**Leaves**	
NDF %		**FTIR**	**NIR**	**FTIR**	**NIR**	**FTIR**	**NIR**
All Data	n	181	181	89	89	92	92
	R^2(calib)	0.92	0.89	0.84	0.91	0.85	0.86
	R^2 (CV)	0.73	0.77	0.55	0.75	0.58	0.54
	MSE(calib)	8.24	11.29	8.83	5.08	8.04	7.21
	MSE(CV)	27.66	23.56	25.45	13.99	22.08	24.27
Outliers Removed	n	171	171	84	84	87	87
	R^2(calib)	0.94	0.86	0.98	0.91	0.83	0.92
	R^2 (CV)	0.72	0.77	0.53	0.77	0.61	0.71
	MSE(calib)	6.27	14.16	1.00	5.05	9.07	4.18
	MSE(CV)	28.77	23.64	26.33	13.59	20.89	14.81
Training Data set	n	129	129	63	63	66	66
	R^2(calib)	0.89	0.87	0.90	0.92	0.80	0.82
	R^2 (CV)	0.67	0.76	0.24	0.75	0.47	0.56
	MSE(calib)	11.29	12.99	6.23	4.64	10.66	9.59
	MSE(CV)	34.92	23.65	47.72	14.24	28.24	23.30
Test Data set	n	42	42	21	21	21	21
	R^2(pred)	0.56	0.70	0.51	0.90	0.84	0.76
	MSE(pred)	40.93	32.68	16.41	6.15	8.88	9.00
	RPD	2.33	2.37	2.54	3.40	2.28	2.31
	RMSECV	4.34	4.25	2.95	2.25	3.32	3.09

4.2.3 Ash

Ash showed mixed results with unexpected low R^2 prediction for FTIR in all and stems, whilst a good result in leaves. NIR predictions performed well across all tissue types. MSE and RMSECV were low in both methods with NIR slightly lower than FTIR. RPD was above 2 for both prediction methods.

First, R^2(calib) was excellent for both methods and all tissue types. FTIR had its highest value across stems and leaves with 0.96 each, and slightly lower 0.91 for all. NIR had its highest value 0.97 L for leaves and not far under was all and stems with 0.94 for each.

The R^2(CV) was impressive for NIR with all 0.89 and leaves 0.92 having excellent values for quality predictions. Stems 0.73, whilst just under excellent will be useful for approximate quality prediction. FTIR had two good middle range values of 0.60 A and 0.59 L in all and leaves. FTIR stems, 0.47 S was just under the lower limit of 0.51 for good prediction.

Interestingly, the R^2(calib) between all data and outliers removed, were all the same but one, stems NIR, 0.8. Therefore, every FTIR and all and stems for NIR were excellent. The R^2(CV) were similar to all data also, 0.61 A, 0.44 S and 0.58 L for FTIR and 0.90 A, 0.69 S and 0.92 L for NIR. NIR all and leaves were still excellent and slightly decreased in stems. FTIR still provided good R^2(CV) for all and leaves but stems decreased further, still being useless for quality prediction.

Training set data had slightly lower R^2(calib), now only three values were above excellent, 0.91 A NIR, 0.90 S and 0.93 L FTIR. The rest of the values were above 0.8, showing good results, 0.87 A FTIR and 0.81 S and 0.88 L NIR. The R^2(CV) was slightly lower with most significant drop in FTIR stems decreasing from 0.44 to 0.22. NIR still had two excellent values in all and leaves, 0.84A 0.88L. The rest were in the good range.

The test data set had mixed R^2(pred) results, 0.55 A, 0.33 S and 0.73 L for FTIR and 0.86 A, 0.79 S and 0.90 L NIR. Clearly, the FTIR values for all and stems showed poor performance, but leaves showed good results. NIR had two good results and one excellent for leaves, making leaves the highest R^2 for both methods.

MSE(calib) for all data set and outliers removed are very similar across all tissue and methods, 0.47 A, 0.16 S and 0.27 L for FTIR all and 0.48 A, 0.16 S and 0.16 L FTIR outliers removed, as well as 0.31 A, 0.23 S and 0.18 L NIR all and 0.29 A, 0.75 S and 0.16 L NIR outliers removed. The only significant difference is in NIR stems increasing from 0.23 to 0.75. MSE(CV) are also very similar between all data and outliers removed.

The MSE(calib) for training set increased from the outliers removed except for NIR stems which decreased from 0.75 to 0.74. MSE(pred) for the test set increased more dramatically for FTIR prediction, 2.75 A, 2.10 S and 1.24 L compared with the small increase in NIR 0.66 A, 0.75 S and 0.87 L.

Although the R^2 prediction is lower than expected for FTIR all and stems, the model is still above RPD 2, 2.10 A and 2.13 S. Only slightly higher is NIR stems, 2.24 S. FTIR has one excellent RPD in leaves, 3.00 whilst NIR has two 3.09 A all and significantly higher, 3.96 L leaves. Leaves has the best model for Ash with both highest R^2(pred) and RPD for both methods. RMSECV is close to 1 for FTIR and between 0.62 and 0.87 for NIR which is minimal.

Table 13: Coefficient of determinations R2, Mean Square Error (MSE) and Relative Performance Deviation (RPD) for Fournier Transformed Infrared (FTIR) and Near Infrared (NIR) Ash %, calibrations (calib) and cross-validation (CV) for stems, leaves and stems plus leaves (All) using all the data, the data after removing the outliers, a training data set (75% of data) and a test data set (25% of data).

		All		**Stems**		**Leaves**	
Ash %		**FTIR**	**NIR**	**FTIR**	**NIR**	**FTIR**	**NIR**
All Data	n	181	181	89	89	92	92
	R^2(calib)	0.91	0.94	0.96	0.94	0.96	0.97
	R^2 (CV)	0.60	0.89	0.47	0.73	0.59	0.92
	MSE(calib)	0.47	0.31	0.16	0.23	0.27	0.18
	MSE(CV)	2.05	0.55	1.99	1.02	2.42	0.45
Outliers Removed	n	171	171	84	84	87	88
	R^2(calib)	0.91	0.94	0.96	0.80	0.96	0.97
	R^2 (CV)	0.61	0.90	0.44	0.69	0.58	0.92
	MSE(calib)	0.48	0.29	0.16	0.75	0.25	0.16
	MSE(CV)	2.09	0.53	2.14	2.27	2.61	0.51
Training Data set	n	129	129	63	63	66	66
	R^2(calib)	0.87	0.91	0.90	0.81	0.93	0.88
	R^2 (CV)	0.52	0.84	0.22	0.63	0.57	0.88
	MSE(calib)	0.67	0.49	0.38	0.74	0.45	0.22
	MSE(CV)	2.42	0.86	2.95	1.41	2.93	0.58
Test Data set	n	42	42	21	21	21	21
	R^2(pred)	0.55	0.86	0.33	0.79	0.73	0.90
	MSE(pred)	2.75	0.66	2.10	0.75	1.24	0.87
	RPD	2.10	3.09	2.13	2.24	3.00	3.96
	RMSECV	1.09	0.73	0.93	0.87	0.81	0.62

4.2.4 CP

The crude protein regression showed poor results for FTIR and excellent results for NIR. The RPD for FTIR was between 2.03 and 2.12, with NIR having large values between 3.79 and 5.58.

Starting with the R^2(calib) for all data, all but one result show excellent values. FTIR had 0.91 A, 0.94 S and 0.89 L and slightly better was NIR with 0.97 A, 0.96 S and 0.98 L. R^2(calib) for outliers removed were very similar with little change for FTIR 0.91 A, 0.95 S and 0.83 L, and a slight decrease for NIR in stems, 0.97 A, 0.84 S and 0.98 L. The

R^2(CV) for FTIR 0.54 A, 0.30 S and 0.32 L, are dramatically lower than NIR, 0.94 A, 0.89 S and 0.94 L. Only all in FTIR had a high enough R^2(CV) to be considered useful, with stems and leaves having lower values than 0.51. NIR, on the other hand, reported all values above 0.74, making them all excellent. The R^2(CV) does not improve for FTIR in stems and leaves when outliers were removed, however all increased slightly to 0.57. NIR results, on the other hand, were still all well above 0.74, maintaining excellent values.

FTIR shows good R^2(calib), 0.81 A, 0.89 S and 0.89 L in the training set, just falling short of excellent for stems and leaves. All the NIR R^2(calib) were excellent, 0.95 A, 0.93 S and 0.97 L with leaves maintaining the highest value. The R^2(CV) drops FTIR all below 0.51 with 0.43 A, 0.27 S and 0.38 L showing poor results across all tissue types. There is no significant change to NIR R^2(CV), resulting in 0.93 A, 0.83 S and 0.93 L.

Test data R^2(pred) is poor for FTIR with the highest values in all and lowest in leaves, 0.57 A, 0.52 S and 0.20 L. The prediction for NIR was excellent with a slight drop for all and leaves, whilst stems remained the same as the training set, 0.91 A, 0.93 S and 0.95 L.

The MSE(calib) in all data, 0.78 A, 0.19 S and 0.89 L for FTIR and 0.28 A, 0.13 S and 0.13 L for NIR, was similar to that in outliers removed, 0.80 A, 0.17 S and 1.40 L for FTIR and 0.28 A, 0.14 S and 0.15 L for NIR. The only significant difference was seen in FTIR leaves increasing from 0.89 to 1.40 in outliers removed. MSE(CV) is higher in FTIR than NIR by a lot with the highest value in leaves, 3.86 A, 2.16 S and 5.40 L. The highest MSE(CV) for NIR was in all, significantly smaller than in FTIR, 0.51 A, 0.35 S and 0.45 L. These results are also very similar to those in outliers removed MSE(CV), 3.78 A 2.30 S and 5.79 L for FTIR and 0.51 A, 0.35 S and 0.45 L for NIR. MSE(calib) in the training data set, are larger for most values, with the exception of FTIR leaves decreasing from 1.40 to 1.26. The rest are 1.80 A and 0.30 S for FTIR and 0.39 A, 0.19S and 0.20 L for NIR. FTIR all increased greatly, having the highest MSE(calib) across all tissue types. The largest value of MSE(calib) for NIR is also in the all tissue group.

The MSE(CV) for FTIR in the training data set increases greatly for all, but decreases slightly for stems and leaves, 5.37 A, 1.89 S and 5.71 L. The NIR MSE(CV) only increased for all and leaves but decreased in stems, 0.61 A, 0.47 S and 0.5 L.

MSE(pred) for the test data is large for FTIR results and the largest for FTIR leaves, 2.24 A, 2.22 S and 3.80 L. The largest MSE(pred) for NIR was in all almost double stems and leaves, 0.79 A, 0.27 S, 0.4 L.

RPD across all methods and tissues is above 2 with the highest in all for FTIR and leaves for NIR. The lowest value was in stems for both FTIR and NIR, 2.12 A, 2.03 S and 2.06 for FTIR and 4.12 A, 3.79 S and 5.58 L for NIR. RMSECV was close to 1 for FTIR and below 1 for NIR which is minimal.

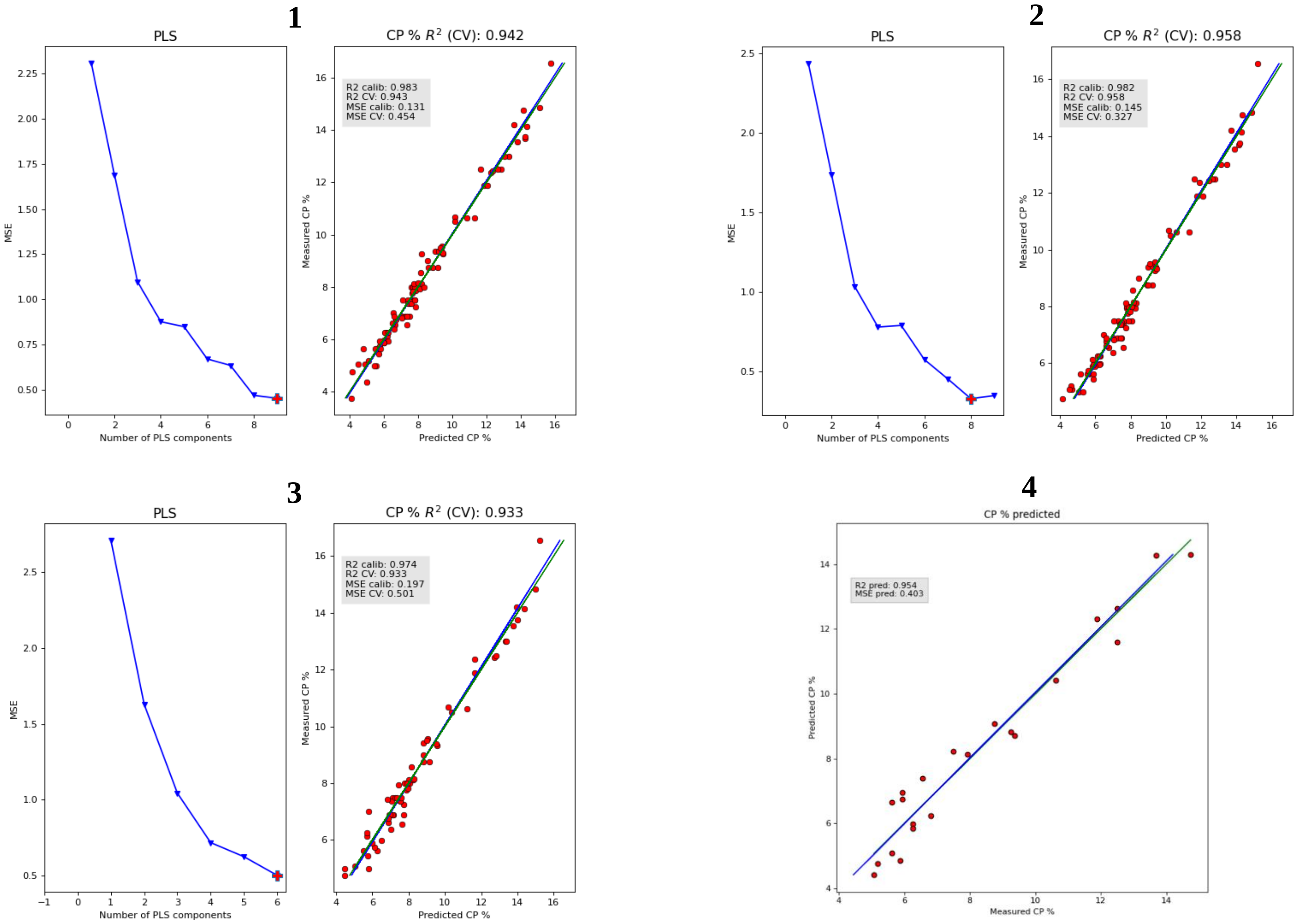


Figure 18: PLSR calibration and prediction results using NIR for CP. 1: All Data, 2: Outliers Removed, 3: Training Data Set, 4 Test Data Set.

Table 14: Coefficient of determinations R2, Mean Square Error (MSE) and Relative Performance Deviation (RPD) for Fournier Transformed Infrared (FTIR) and Near Infrared (NIR) Dietary crude protein (CP %) calibrations (calib) and cross-validation (CV) for stems, leaves and stems plus leaves (All) using all the data, the data after removing the outliers, a training data set (75% of data) and a test data set (25% of data).

		All		**Stems**		**Leaves**	
CP %		**FTIR**	**NIR**	**FTIR**	**NIR**	**FTIR**	**NIR**
All Data	n	181	181	89	89	92	92
	R^2(calib)	0.91	0.97	0.94	0.96	0.89	0.98
	R^2 (CV)	0.54	0.94	0.30	0.89	0.32	0.94
	MSE(calib)	0.78	0.28	0.19	0.13	0.89	0.13
	MSE(CV)	3.86	0.51	2.16	0.35	5.40	0.45
Outliers Removed	n	171	171	84	84	87	87
	R^2(calib)	0.91	0.97	0.95	0.84	0.83	0.98
	R^2 (CV)	0.57	0.93	0.28	0.84	0.29	0.95
	MSE(calib)	0.80	0.28	0.17	0.14	1.40	0.15
	MSE(CV)	3.78	0.56	2.30	0.50	5.79	0.33
Training Data set	n	129	129	63	63	66	66
	R^2(calib)	0.81	0.95	0.89	0.93	0.89	0.97
	R^2 (CV)	0.43	0.93	0.27	0.83	0.38	0.93
	MSE(calib)	1.80	0.39	0.30	0.19	1.26	0.20
	MSE(CV)	5.37	0.61	1.89	0.47	5.71	0.50
Test Data set	n	42	42	21	21	21	21
	R^2(pred)	0.57	0.91	0.52	0.93	0.20	0.95
	MSE(pred)	2.24	0.79	2.22	0.27	3.80	0.40
	RPD	2.12	4.12	2.03	3.79	2.06	5.58
	RMSECV	1.40	0.70	0.88	0.46	1.39	0.50

4.2.5 ADF%

All data R^2(calib) results were good and excellent for FTIR and NIR. FTIR had one excellent value in all and good values for stems and leaves, 0.93 A, 0.86 S and 0.83 L. NIR had 2 excellent values in all and leaves, and a good value just below excellent for stems, 0.90 A, 0.88 S and 0.92L.

After removing outliers, the R^2(calib) increased in most cases. The FTIR results improved the stems value making it excellent and highest value between either method but did not increase leaves enough to pass above 0.9, 0.95 A, 0.98 S and 0.84 L. The NIR results dropped from excellent to good in all, whereas stems increased from good to excellent. Leaves had a slight increase, 0.88 A, 0.92 S and 0.94 L.

The R^2(CV) in all data was excellent for both methods in all, and good for approximate quality prediction in both methods for stems and FTIR for leaves. The R^2(CV) was poor for NIR leaves with a value slightly below 0.51, 0.79 A, 0.63 S and 0.58 L for FTIR and 0.85 A, 0.71 S and 0.45 L for NIR. After outliers are removed, R^2(CV) for NIR values are all excellent whilst only all was excellent for FTIR. The values for stems and leaves for FTIR were good, 0.78 A, 0.61 S and 0.59 L for FTIR and 0.82 A, 0.83 S and 0.81 L for NIR.

The training data set had two excellent values for R^2(calib) which were 0.90 A and 0.94 S for NIR. NIR leaves was good, 0.86 just below the limit of excellent. The results for FTIR were very close to excellent with 0.89 A, 0.89 S and 0.88 L just falling under. Similarly, to R^2(calib), R^2(CV) had two excellent values 0.86 A and 0.79 S for NIR. The other NIR value was good 0.63 L. FTIR had good R^2(CV) for all (0.70) and stems (0.52) but a poor value for leaves (0.31).

The test set R^2(pred) values were notably lower than all previous except for NIR leaves. FTIR had two good values in all and leaves but a poor value in stems, 0.71 A, 0.53 S and 0.75 L. NIR had good values in all and stems and one excellent value in leaves, 0.78 A, 0.77 S and 0.92 L. ADF has its highest R^2(pred) in leaves for both methods.

MSE(calib) for all data is similar between all and stems for FTIR but drops for leaves. A similar pattern is seen for NIR where MSE(calib) is large for all and stems and also drops for leaves, 6.89 A, 6.95 S and 0.92 L for FTIR and 9.90 A, 6.12 S and 2.25 L for NIR. MSE(CV) is far higher than MSE(calib), almost 3 times higher for FTIR. The highest values are in all and lowest seen in leaves, 20.63 A, 18.34S and 4.43 L for FTIR and 9.90 A, 6.12 S and 2.25 L for NIR.

For most results MSE(calib) decreased after outliers were removed in both methods. The largest value was in all again, this time being NIR instead of FTIR. The lowest value was in FTIR stems, slightly lower than NIR leaves, 4.96 A, 0.86 S and 4.39 L for FTIR and 11.56 A, 3.71 S and 1.28 L for NIR. Although MSE(calib) was lower for FTIR all and stems, the MSE(CV) was larger for FTIR than NIR. FTIR leaves was very similar to the all data value. NIR stems and leaves were noticeably lower than in all data, 22.08 A, 19.31 S and 11.14 L for FTIR and 17.67 A, 8.02 S and 4.04 L for NIR.

The training set MSE(calib) is lower than outliers removed for most values, 11.16 A, 5.29 S and 2.62 L for FTIR and 9.63 A, 2.93 S and 2.94 L for NIR. The MSE(CV) is the opposite, with four of six values increasing, 2.32 A, 23.58 Sand 15.68 L for FTIR and 14.25 A, 10.25 S and 7.52 L for NIR. The values that decrease were both FTIR and NIR all, dropping from 22.08 and 17.67 to 2.32 and 14.25 receptively. The test data set had large MSE(pred) values for FTIR with an extremely large value for FTIR all. NIR shared a very large MSE(pred) for all but the values for stems and leaves were small, 31.57 A, 23.97 S and 10.20 L for FTIR and 21.04 A, 0.78 S and 1.98 L for NIR. All results showed RPD greater than 2, although NIR was higher than FTIR in each tissue type, 2.52 A, 2.23 S and 2.44 L fir FTIR and 2.80 A, 3.36 S and 2.82 L for NIR. The RPD for all and leaves were similar however, stems had the lowest value for FTIR and the highest value for NIR. RMSECV was highest for all and lowest for leaves. The largest value was seen in NIR all but stems and leaves were lower than FTIR, 3.98 A, 3.16 S and 2.14 L for FTIR and 9.91 A, 2.05 S and 1.65 L for NIR.

Table 15: Coefficient of determinations R2, Mean Square Error (MSE) and Relative Performance Deviation (RPD) for Fournier Transformed Infrared (FTIR) and Near Infrared (NIR) Acid Detergent Fiber (ADF %) calibrations (calib) and cross-validation (CV) for stems, leaves and stems plus leaves (All) using all the data, the data after removing the outliers, a training data set (75% of data) and a test data set (25% of data).

		All		**Stems**		**Leaves**	
ADF %		**FTIR**	**NIR**	**FTIR**	**NIR**	**FTIR**	**NIR**
All Data	n	181	181	89	89	92	92
	R^2(calib)	0.93	0.90	0.86	0.88	0.83	0.92
	R^2 (CV)	0.79	0.85	0.63	0.71	0.58	0.45
	MSE(calib)	6.89	9.90	6.95	6.12	4.43	2.25
	MSE(CV)	20.63	15.19	18.34	14.62	11.09	14.54
Outliers Removed	n	171	172	84	84	87	87
	R^2(calib)	0.95	0.88	0.98	0.92	0.84	0.94
	R^2 (CV)	0.78	0.82	0.61	0.83	0.59	0.81
	MSE(calib)	4.96	11.56	0.86	3.71	4.39	1.28
	MSE(CV)	22.08	17.67	19.31	8.02	11.14	4.04
Training Data set	n	129	129	63	63	66	66
	R^2(calib)	0.89	0.90	0.89	0.94	0.88	0.86
	R^2 (CV)	0.70	0.86	0.52	0.79	0.31	0.63
	MSE(calib)	11.16	9.63	5.29	2.93	2.62	2.94
	MSE(CV)	2.32	14.25	23.58	10.25	15.68	7.52
Test Data set	n	42	42	21	21	21	21
	R^2(pred)	0.71	0.78	0.53	0.77	0.75	0.92
	MSE(pred)	31.57	21.04	23.97	0.78	10.20	1.98
	RPD	2.52	2.80	2.23	3.36	2.44	2.82
	RMSECV	3.98	9.91	3.16	2.05	2.14	1.65

4.2.6 *In Vitro* DOMD

The In Virto DOMD regression shows good results with high R^2, large RPD and relatively small error for FTIR and NIR methods.

The R^2(calib) for all data was excellent for both FTIR an NIR methods, with FTIR having larger values for all and stems, 0.95 A, 0.97 S and 0.92 L for FTIR and 0.92 A, 0.94 S and 0.95 L for NIR. The R^2(CV) was equally impressive with excellent values for all but NIR leaves. Even this low value compared to the other results is just under the cut off for excellent. The highest values were all and stems for NIR and all for FTIR, 0.86 A, 0.80 S and 0.78 L for FTIR, and 0.88 A, 0.88 S, 0.71 L for NIR.

Once outliers had been removed R^2(calib) increased for most values and decreased by 0.01 in NIR stems. The largest FTIR R^2(calib) was in stems, whereas NIR had its largest result in leaves, 0.96 A, 0.99 S and 0.94 L for FTIR and 0.92 A, 0.93 S and 0.97 L for NIR. The R^2(CV) for outliers removed was very similar to that in all data with only significant change in leaves. FTIR decreased from 0.78 to 0.72 making it no longer excellent for leaves. The opposite occurred in NIR where the value increased from 0.71 to 0.89 making it excellent and the highest value across all tissue types.

The excellent results continue for R^2(calib) in the training set, with excellent results for FTIR and NIR. The highest value for FTIR and NIR was in stems, 0.92 A, 0.94 S and 0.91 L for FTIR and 0.90 A, 0.96 S and 0.94 L for NIR. R^2(CV) for the training set had mostly excellent results and one good result for FTIR stems, 0.71. The R^2(CV) was higher for NIR than FTIR in each tissue type, 0.75 A, 0.71 S and 0.75 L for FTIR and 0.83 A, 0.89 S and 0.77 L for NIR.

The R^2(pred) from the test set were good with only one value dropping below 0.7. FTIR had two good R^2 values in all and stems but a poor result in leaves. NIR had good results across all tissues, with the highest in all. NIR had higher R^2(pred) in all and leaves but slightly lower than FTIR in stems, 0.84 A, 0.82 S and 0.64 L for FTIR and 0.88 A, 0.77 S and 0.80 L for NIR.

MSE(calib) for all data is the largest in all for FTIR and NIR compared to stems and leaves. The smallest MSE(calib) for FTIR was in stems, whilst the smallest NIR value was in leaves, 2.69 A, 0.79 S and 1.75 L for FTIR and 4.38 A, 1.94 S and 1.12 L for NIR.

MSE(CV) was also highest in all for both methods. FTIR is larger than NIR in all and stems but smaller for leaves. The smallest value was stems NIR which was much lower than the rest of the results, 7.74 A, 5.85 S and 5.01 L for FTIR and 6.29 A, 3.50 S and 6.24 L for NIR.

MSE(calib) for outliers removed decreases slightly for FTIR across all tissue types compared with all data, whilst the opposite occurs for NIR with a slight increase in all and stems. The only notable difference was in leaves NIR which decreases from 1.12 to 0.54 and FTIR stems decrease from 0.75 to 0.36. The full set were 2.10 A, 0.36 S and 1.41 L for FTIR and 4.46 A, 2.20 S and 0.54 L for NIR. MSE(CV) in outliers removed was very similar to all data except for NIR leaves which drops dramatically from 6.24 to 2.19, 7.76 A, 5.54 S and 6.01 L for FTIR and 7.04 A, 3.67 S and 2.19 L for NIR. MSE(calib) for training data set were larger than outliers removed except for NIR stems which dropped from 2.20 to 1.47. The largest values for FTIR and NIR were in all, much higher than the other values in stems and leaves. The values in stems and leaves were similar, ranging from 1.31 to 1.97. The full set are 4.12 A, 1.61 S and 1.97 L for FTIR and 5.48 A, 1.47 S and 1.31 L for NIR. MSE(CV) values were also larger for most with exception to stems NIR and leaves FTIR which were slightly smaller. The largest increase was in all FTIR and leaves NIR, 13.84 A, 8.4 S and 5.38 L for FTIR and 9.44 A, 3.55 S and 5.11 L for NIR.

MSE(pred) continues the trend with FTIR having larger values than NIR. The largest FTIR values was in all and its lowest in stems. This was slightly different to NIR, with its largest value in all and smallest in leaves. The training set was higher than every other data set with 9.41 A, 6.03 S and 8.15 L for FTIR and 5.78 A, 4.79 S and 2.23 L for NIR.

RPD was higher than 2 for FTIR leaves and higher than 3 for FTIR all and stems in addition to every NIR result. The highest RPD was in stems for both methods, where NIR was higher than FTIR. FTIR was only higher than NIR in all, 3.19 A, 3.33 S and 2.47 L for FTIR and 3.11 A, 3.60 S and 3.55 L for NIR. The RMSECV was largest for all, where FTIR and NIR were similar. Stems results were also similar between methods. FTIR was higher than NIR for leaves, 2.34 A, 1.66 S and 1.89 L for FTIR and 2.36 A, 1.53 S and 1.25 L for NIR.

Table 16: Coefficient of determinations R2, Mean Square Error (MSE) and Relative Performance Deviation (RPD) for Fournier Transformed Infrared (FTIR) and Near Infrared (NIR) In Vitro Dry Organic Matter Digestibility (DOMD %) calibrations (calib) and cross-validation (CV) for stems, leaves and stems plus leaves (All) using all the data, the data after removing the outliers, a training data set (75% of data) and a test data set (25% of data).

		All		**Stems**		**Leaves**	
***In Vitro* DOMD %**		**FTIR**	**NIR**	**FTIR**	**NIR**	**FTIR**	**NIR**
All Data	n	181	181	89	89	92	92
	R^2(calib)	0.95	0.92	0.97	0.94	0.92	0.95
	R^2 (CV)	0.86	0.88	0.80	0.88	0.78	0.71
	MSE(calib)	2.69	4.38	0.79	1.94	1.75	1.12
	MSE(CV)	7.74	6.29	5.85	3.50	5.01	6.24
Outliers Removed	n	171	84	84	84	87	88
	R^2(calib)	0.96	0.92	0.99	0.93	0.94	0.97
	R^2 (CV)	0.86	0.87	0.82	0.88	0.72	0.89
	MSE(calib)	2.10	4.46	0.36	2.20	1.41	0.54
	MSE(CV)	7.76	7.04	5.54	3.67	6.01	2.19
Training Data set	n	129	129	63	63	66	66
	R^2(calib)	0.92	0.90	0.94	0.96	0.91	0.94
	R^2 (CV)	0.75	0.83	0.71	0.89	0.75	0.77
	MSE(calib)	4.12	5.48	1.61	1.47	1.97	1.31
	MSE(CV)	13.84	9.44	8.40	3.55	5.38	5.11
Test Data set	n	42	42	21	21	21	21
	R^2(pred)	0.84	0.88	0.82	0.77	0.64	0.80
	MSE(pred)	9.41	5.78	6.03	4.79	8.15	2.23
	RPD	3.19	3.11	3.33	3.60	2.47	3.55
	RMSECV	2.34	2.36	1.66	1.53	1.89	1.25

4.3 Post Prediction Results

4.3.1 Correlation Coefficient Results

Table 17: Correlation coefficients between analytical values for All (Stem and leaves).

All	DM %	Ash %	CP %	NDF %	ADF %	In vitro DOMD %
Ash %	-0.108					
CP %	0.133	0.494				
NDF %	-0.200	-0.036	-0.208			
ADF %	-0.210	-0.139	-0.365	0.949		
In vitro DOMD %	0.365	-0.165	0.225	-0.892	-0.906	
ME	0.365	-0.163	0.226	-0.892	-0.907	1

r > |0.146| p< 0.05

The correlation coefficient value for DM % had two not-significantly greater than zero values, Ash % -0.108 and CP % 0.133, as well as four weak correlations, NDF % -0.200, ADF % -0.210, DOMD % 0.365 and ME 0.365. Ash % has a medium correlation to CP % 0.494, two correlations not significantly greater than zero, NDF % -0.036 and ADF % -0.139. In addition, Ash % has a weak correlation with DOMD % -0.165 and ME -0.163. CP % has one not significantly greater than zero correlation with DM % 0.133 and four weak correlations with NDF % -0.208, ADF % -0.365, DOMD 0.225 and ME 0.226. The largest correlation is with Ash % 0.494. NDF % has one not significantly greater than zero correlation with Ash % -0.139. There are two weak correlations with DM % -0.200 and CP -0.208. Significant correlation occurs between NDF % and ADF % 0.949, DOMD -0.892 and ME -0.892. ADF % has one not significantly greater than zero correlation with Ash % -0.20, two weak correlations with DM % -0.210 and CP -0.365. There are large correlations between ADF with NDF % 0.949, DOMD -0.906 and ME -0.907. DOMD % has low correlation with Ash % -0.165, CP % 0.225 and DM % 0.365. DOMD % shares strong correlation with NDF % -0.892 and ADF % -0.906. ME has low correlation to Ash % -0.630, CP % 0.225 and DM 0.365. There is strong correlation between NDF -0.892, ADF -0.907 and absolute correlation with DOMD % 1.

The correlations of interest are between ME and DOMD % as well as the correlation between DOMD and both ADF and NDF. The ME-DOMD % correlation can be explained by the formula ME=16.3xDOMD %. The high correlation between NDF % and ADF % is due to their similar properties and how they are determined. The relationship between DOMD and NDF/ADF suggests the higher the NDF or ADF the lower the DOMD will be.

Table 18: Correlation coefficients between analytical values for Stems and Leaves.

Stems	DM %	Ash %	CP %	NDF %	ADF %	In vitro DOMD %
Ash %	-0.232					
CP %	-0.040	0.426				
NDF %	-0.045	0.093	0.317			
ADF %	-0.098	0.134	0.303	0.974		
In vitro DOMD %	0.337	-0.425	-0.363	-0.846	-0.884	
ME	0.337	-0.423	-0.362	-0.846	-0.885	1

r > |0.208| p< 0.05

Leaves	DM %	Ash %	CP %	NDF %	ADF %	In vitro DOMD %
Ash %	-0.082					
CP %	0.107	0.481				
NDF %	-0.200	0.218	0.333			
ADF %	-0.128	-0.022	0.096	0.850		
In vitro DOMD %	0.360	-0.556	-0.374	-0.724	-0.647	
ME	0.361	-0.556	-0.373	-0.724	-0.647	1

r >|0.205| p< 0.05

The correlation coefficients between analytical values for stems or leaves seen in Table 17 were very similar to those in Table 18, with the exception of some CP % correlations. The correlation coefficient for leaves and stems for NDF % and ADF % changed signs from negative -0.208 leaves and -0.365 stems to positive 0.333, 0.096 leaves, and 0.317, 0.303 stems respectively. The opposite occurred between CP % and both DOMD % and ME changing from positive 0.225 or 0.226 in all, to -0.363, -0.362 stems and -0.374, -0.373 leaves respectively. This occurrence is due to the two different clusters of data for leaves and stems.

4.3.2 Post prediction Observed vs Predicted Statistics

Tables 19, 20 and 21 are the product of post prediction statistics. The observed and predicted values are simply the x and y data for each sample used in the large dataset prediction with 171 samples. Figures 19-21 show the one size fits all model plots (Total) of CP, NDF and ME for FTIR and NIR.

The observed (wet chemical) values are slightly different for some species between FTIR and NIR because the outliers removed were different in each method, resulting in a unique mean. Only CP, NDF and ME were investigated in this section as these analytes are most prevalent in the literature.

4.3.2.1 CP

The bias was low in NIR ranging from 0.004 Salix, to 0.28 in Pseudopanax. All had a low bias, -0.011 whilst leaves and stems were slightly larger, -0.07 and 0.06 respectively. Overall, there were slightly more under predicted than over predicted values. FTIR had a relatively larger bias rearranging from 0.08 in Coprosma to 0.80 in Pseudopanax. All, leaves and stems were also larger (0.09, -0.14 and 0.32). All was still the lowest between tissue types like in NIR. The p-value paired t-test did not show any significance ($p<0.05$) between the observed and predicted results in NIR. The highest values were Salix 0.98 and Griselinia 0.92.

In FTIR, Stems, Hoheria, and Pseudopanax had p values below 0.05, indicating there was a significant difference between observed and predicted values. The largest p-values were in Coprosma 0.69, and Mahoe 0.61.

The mean prediction error (MPE) for NIR was similar, with the largest 0.85 in Salix and lowest 0.54 in Pittosporum. All and Leaves were similar, 0.70 and 0.74, slightly higher than Stems, 0.65. Mahoe, Pseudopanax, and Hoheria had values between 0.59 and 0.63. Coprosma and Hoheria had the joint second highest value 0.78.

There was a large range of MPE values in FTIR, with the largest in Salix, 1.6 and the smallest in Mahoe, 0.94. There was a difference between All, Leaves and Stems, 1.39, 1.57 and 1.20 compared with NIR with FTIR being much larger, although the size order was maintained. The rest of the species had values between 1.20 (Coprosma) and 1.51 (Hoheria).

The RPE across the NIR results have a small range, from 0.06 in Hoheria to 0.16 in Griselinia. Stems had the largest error of the three tissue types of 0.13, whilst All and Leaves are slightly lower 0.10 and 0.09. Mahoe, Pittosporum and Salix have similar values between 0.07-0.10, whilst Coprosma and Pseudopanax were slightly larger 0.12 and 0.14.

FTIR had a larger RPE range of 0.21, about double compared to NIR. The two largest values were in Pseudopanax 0.33 and Griselinia 0.30. FTIR again followed the same trend for the three tissue types with Stems the largest, Leaves the smallest and All between both. Coprosma, Pittosporum and Salix were in the middle range 0.18-0.26 and Griselinia was the second lowest 0.15.

CCC values between 0.81 and 1 are considered excellent and suggest having an almost perfect prediction (Lin, 1989). All of the values for NIR were greater than 0.81. The highest value 0.97 was shared by All, Hoheria, Mahoe and Salix. The lowest two values were 0.89 Griselinia and 0.90 Coprosma.

The CCC for FTIR are lower with the largest value being 0.91 shared between Salix and Mahoe. The other values that are considered excellent include All, Leaves and Hoheria. Stems, Coprosma, Pittosporum and Pseudopanax had significant prediction between 0.61-0.80. Griselinia was the lowest value, 0.55, suggesting only moderate prediction.

R^2 for NIR was excellent for All, Leaves, Hoheria, Mahoe and Salix, $R^2>0.9$. The rest, Stems, Coprosma, Griselinia, Pittosporum and Pseudopanax were above $0.8<R^2$ and considered a good prediction. All 0.94 having and excellent result suggests one model is adequate.

The results were less convincing for FTIR, with no excellent values, however, Mahoe was very close 0.89. The values with good prediction were All, Leaves, Hoheria, Mahoe and Salix. Leaves 0.71 and Hoheria 0.74 were on the lower end. Moderate predictions included Stems, Coprosma and Pseudopanax. Finally, poor prediction was seen for Pittosporum 0.43 and Griselinia 0.31.

4.3.2.2 NDF

NDF has a larger bias overall for NIR and FTIR compared with CP and ME. NIR has equal number of over and underpredicted values, with a minimum bias of 0.11 for All and a maximum bias of 3.37 for Pseudopanax. All and leaves both have a bias of 0.74 whilst stems had a lower bias -0.54, underpredicting compared to the observed value. Griselinia had the second highest bias, -0.82. All the other species were between -0.50<B<0.40.

FTIR also had an equal number of under and overpredicted values, with a lower max bias in Pseudopanax (2.06) compared with NIR, but a larger bias for most other species including Coprosma (1.51), Griselinia (-1.66), Pittosporum (-1.37) and Salix (0.99). Hoheria (-0.75) and Mahoe (0.23) were lower than NIR in comparison. The smallest bias was in All -0.03, significantly smaller than the rest of FTIR. Leaves (0.46) had a smaller bias than stems (-0.53) which differed from NIR.

The P-value paired t-test detected that Griselinia had observed and predicted results that were significantly different across its results. All of the other p-values were above 0.05 but Pseudopanax was low, 0.07. FTIR had two p-value results lower than 0.05, 0.02 for Coprosma and Pittosporum. This means there was a significant difference between the observed and predicted results.

MPE for NDF was notably higher than CP and ME but this is because NDF values are larger. The largest errors in NIR were Pseudopanax (7.87) and Salix (6.53). The rest of the species were between 1.21 for Mahoe and 3.69 for Pittosporum. All and leaves had similar MPE, 4.24 and 4.89 compared to stems 3.45. FTIR had a lower max value seen in Griselinia 6.61 and second highest, 4.85 for Pseudopanax. The rest of the species were between 2.37 (Mahoe) and 3.72 (Coprosma). Interestingly, all, leaves and stems had very similar MPE in FTIR, 4.33, 4.30 and 4.36.

The RPE was relatively low in both NIR and FTIR both sharing the largest values for Pseudopanax 0.29 and 0.18 respectively. The rest of the values were very similar between methods. The lowest value was in Mahoe, 0.02 NIR and 0.04 FTIR. All, leaves and stems were 0.10, 0.14 and 0.07 for NIR and 0.10, 0.12 and 0.09 for FTIR.

Similar to CP, there are many results with CCC >0.81, suggesting excellent predictions in both NIR and FTIR. The excellent NIR values include All, Leaves, Coprosma, Griselinia, Hoheria, Mahoe and Pittosporum, all above 0.90. Leaves was slightly lower with 0.74 suggesting significant prediction. Pseudopanax and Salix were far lower, 0.43 and 0.32, suggesting poor prediction.

FTIR had fewer excellent results above 0.9, all, Hoheria, Mahoe and Pittosporum but leaves, stems, Coprosma and Pseudopanax were still above 0.81 suggesting excellent predictions. The remaining two species, Griselinia and Salix had CCC of 0.7 suggesting significant prediction.

R^2 was excellent for Griselinia, Hoheria and Mahoe $R^2>0.90$ in NIR, with Mahoe having a very impressive R^2 of 0.99. All, Stems, Coprosma and Pittosporum had good predictions $R^2>0.8$. Leaves had a moderate prediction (0.57). Unsurprisingly, Pseudopanax and Salix gave poor predictions with very low R^2 values 0.23 and 0.10.

FTIR had one excellent R^2, 0.95 for Mahoe. The next two highest R^2 were Pittosporum and Hoheria, 0.87 and 0.86. These three being top was expected from the CCC results as all were above 0.9. Other good predictions included all (0.82), Pseudopanax (0.80), Coprosma (0.71) and leaves (0.70). Moderate prediction included stems (0.68), Griselinia (0.53) and Salix (0.55).

4.3.2.3 ME

ME has a very low bias with more over predicting values for NIR and an equal number of over and underpredicting values for FTIR. The largest and smallest bias for NIR 0.17 (Salix and Mahoe) and 0.02 (Coprosma and Mahoe) were similar in FTIR, 0.18 (Pittosporum) and 0.01 (Pseudopanax). All and Coprosma have the same bias 0.02 in FTIR as in NIR.

The p-value paired t-test found four samples for NIR and three for FTIR less than $p<0.05$, showing observed and predicted results that were significantly different. Leaves, stems and Pittosporum were in both FTIR and NIR, with Salix making up the fourth for NIR. The lowest bias in each method was Pseudopanax. ME had the lowest MPE compared with CP and NDF. The largest errors occurred in the same two species Pseudopanax and Coprosma for NIR (0.99, 0.90) and FTIR (0.93, 0.84). The smallest error was also similar between methods for stems and Pittosporum, 0.01 and 0.02 in NIR, and 0.01 and 0.06 in FTIR. Mahoe and Salix were higher for FTIR (0.79, 0.66) than NIR (0.22, 0.18), whilst Coprosma, Griselinia and Hoheria were similar between methods, 0.90, 0.63, 0.35 in NIR and 0.84, 0.67 and 0.32 in FTIR respectively. All has the largest error compared with stems and leaves.

The RPE for ME is also very small compared to CP and NDF. The largest error was similar between methods 0.07 for Pseudopanax in NIR and 0.08 for Mahoe in FTIR. The smallest error 0.01 NIR and 0.0002 FTIR was in stems. The error for all, leaves, stems and Pittosporum were all below 0.005 across both methods. The rest of the results are still very minimal ranging from 0.01-0.02 NIR and 0.01-0.06 FTIR.

The CCC results are very impressive for both methods with many values above 0.81. In NIR, all, stems, Griselinia, Hoheria, Mahoe and Pittosporum have CCC>0.9, with the largest 0.98 for Mahoe and Pittosporum. Leaves, and Coprosma were also above 0.81 so all of these results suggest excellent, near perfect predictions. Salix was slightly lower, 0.73 but still suggested to have significant prediction. Pseudopanax was the lowest, 0.56, and only suggested moderate prediction.

FTIR had all, stems, Coprosma, Hoheria, Mahoe, Pittosporum and Pseudopanax above 0.9. The largest value was 0.95 for Mahoe and Pseudopanax. Leaves, and Salix were above 0.81 so also had excellent prediction like the previously mentioned species. Griselinia had the lowest CCC with 0.80, suggesting a significant prediction.

The R^2 was excellent for all, Griselinia, Hoheria, Mahoe and Pittosporum for NIR. The best prediction was Mahoe (0.98), closely followed by Pittosporum (0.96). Leaves (0.79), stems (0.85) and Coprosma (0.79) had good R^2 for prediction. Salix showed moderate prediction, 0.6, whilst Pseudopanax was poor with an R^2 of 0.35.

FTIR had three excellent R^2 values Mahoe 0.90, Pittosporum 0.91 and Pseudopanax 0.91. All (0.89), leaves (0.76), stems (0.84), Coprosma (0.87) and Hoheria (0.89) had good R^2 values, all and Hoheria just lower than excellent. The lowest R^2 values were in Salix 0.69 and Griselinia 0.67, making them moderate for quality prediction.

Figure 19-21 displays observed vs predicted results for All, showing species and tissue type with different shapes and colours. The figure makes it easy to see how different species are distributed across each analyte and most obvious, how good the models were. It is clear to see that NIR is best for CP and good for ME. The NDF plot is not as good with two groupings due to stems and leaves. The FTIR model although worse than NIR still produces a reasonable prediction for CP. In NDF and ME FTIR performs similar to NIR with similar R^2 results.

Table 19: Observed and predicted value, bias (paired t-test) Mean prediction error (MPE), Relative Prediction Error (RPE), concordance correlation coefficient (CCC) and coefficient of determination (R2) for the final over NIR and FTIR prediction model for Neutral Detergent Fibre (NDF) for all, leaves or stem samples only, and for each species.

NIR										
NDF %	All	Leaves	Stems	Coprosma	Griselinia	Hoheria	Mahoe	Pittosporum	Pseudopanax	Salix
n	171	86	85	38	22	27	10	38	18	18
Observed	41.13	34.36	47.97	39.70	38.41	43.46	56.02	42.31	27.52	46.81
Predicted	41.24	35.11	47.44	39.29	37.59	43.67	55.74	41.82	30.89	47.20
Bias	0.11	0.74	-0.54	-0.41	-0.82	0.21	-0.28	-0.49	3.37	0.39
P-value paired t-test	0.74	0.16	0.15	0.40	0.03	0.68	0.49	0.46	0.07	0.81
MPE	4.24	4.89	3.45	2.95	1.80	2.53	1.21	3.96	7.87	6.53
RPE	0.10	0.14	0.07	0.07	0.05	0.06	0.02	0.09	0.29	0.14
CCC	0.91	0.74	0.90	0.90	0.98	0.94	0.99	0.90	0.43	0.32
R2	0.82	0.57	0.82	0.82	0.97	0.91	0.99	0.82	0.23	0.10
FTIR										
NDF %	All	Leaves	Stems	Coprosma	Griselinia	Hoheria	Mahoe	Pittosporum	Pseudopanax	Salix
n	171	86	85	34	27	28	10	35	17	20
Observed	41.38	34.47	48.36	39.92	39.01	43.20	56.02	43.04	27.26	46.25
Predicted	41.34	34.93	47.83	41.42	37.34	42.45	56.26	41.67	29.33	47.25
Bias	-0.03	0.46	-0.53	1.51	-1.66	-0.75	0.23	-1.37	2.06	0.99
P- value paired t-test	0.92	0.32	0.26	0.02	0.20	0.21	0.77	0.02	0.08	0.30
MPE	4.33	4.30	4.36	3.73	6.61	3.16	2.37	3.67	4.85	4.16
RPE	0.10	0.12	0.09	0.09	0.17	0.07	0.04	0.09	0.18	0.09
CCC	0.91	0.83	0.82	0.82	0.70	0.92	0.97	0.92	0.84	0.70
R2	0.82	0.70	0.68	0.71	0.53	0.86	0.95	0.87	0.80	0.55

Table 20: Observed and predicted value, bias (paired t-test) Mean prediction error (MPE), Relative Prediction Error (RPE), concordance correlation coefficient (CCC) and coefficient of determination (R2) for the final over NIR and FTIR prediction model for Crude Protein (CP) for all samples, leaves or stem sample only, and for each species.

NIR										
CP %	All	Leaves	Stems	Coprosma	Griselinia	Hoheria	Mahoe	Pittosporum	Pseudopanax	Salix
n	171	88	83	38	28	28	10	38	9	20
Observed	6.90	8.53	5.17	6.71	4.97	10.18	8.15	5.72	4.40	8.10
Predicted	6.89	8.45	5.23	6.66	4.95	10.02	8.04	5.82	4.69	8.09
Bias	-0.01	-0.07	0.06	-0.06	-0.01	-0.15	-0.11	0.10	0.28	-0.005
P- value paired t-test	0.84	0.35	0.42	0.66	0.92	0.20	0.59	0.26	0.17	0.98
MPE	0.70	0.74	0.65	0.78	0.78	0.63	0.59	0.54	0.60	0.85
RPE	0.10	0.09	0.13	0.12	0.16	0.06	0.07	0.09	0.14	0.10
CCC	0.97	0.96	0.93	0.90	0.89	0.97	0.97	0.93	0.92	0.97
R2	0.94	0.93	0.87	0.81	0.83	0.95	0.95	0.89	0.88	0.96
FTIR										
CP %	All	Leaves	Stems	Coprosma	Griselinia	Hoheria	Mahoe	Pittosporum	Pseudopanax	Salix
n	171	85	86	35	27	28	10	34	18	19
Observed	6.75	8.48	5.04	6.75	4.97	10.18	8.15	5.62	4.06	8.06
Predicted	6.84	8.34	5.36	6.84	5.27	9.55	7.98	6.01	4.86	7.84
Bias	0.09	-0.14	0.32	0.08	0.30	-0.63	-0.16	0.39	0.80	-0.22
P- value paired t-test	0.40	0.40	0.01	0.69	0.29	0.02	0.61	0.12	0.01	0.56
MPE	1.39	1.57	1.20	1.20	1.47	1.51	0.94	1.45	1.33	1.60
RPE	0.21	0.18	0.24	0.18	0.30	0.15	0.12	0.26	0.33	0.20
CCC	0.87	0.84	0.73	0.76	0.55	0.83	0.91	0.63	0.61	0.91
R2	0.78	0.71	0.58	0.58	0.31	0.74	0.89	0.43	0.50	0.85

Table 21: Observed and predicted value, bias (paired t-test) Mean prediction error (MPE), Relative Prediction Error (RPE), concordance correlation coefficient (CCC) and coefficient of determination (R2) for the final over NIR and FTIR prediction model for metabolisable energy (ME) for all samples, leaves or stem sample only, and for each species.

NIR										
ME MJ/kg	All	Leaves	Stems	Coprosma	Griselinia	Hoheria	Mahoe	Pittosporum	Pseudopanax	Salix
n	171	84	87	38	27	28	9	35	14	20
Observed	10.20	11.07	9.36	10.60	10.70	9.75	7.94	10.01	11.04	10.19
Predicted	10.22	11.00	9.48	10.62	10.66	9.82	8.10	9.92	11.04	10.36
Bias	0.02	-0.08	0.11	0.02	-0.04	0.07	0.17	-0.09	0.004	0.17
P- value paired t-test	0.50	0.04	0.01	0.80	0.40	0.12	0.05	0.02	0.98	0.03
MPE	0.71	0.21	0.09	0.90	0.63	0.35	0.22	0.15	0.99	0.18
RPE	0.004	0.002	0.001	0.02	0.02	0.01	0.02	0.004	0.07	0.01
CCC	0.95	0.88	0.91	0.88	0.97	0.97	0.98	0.98	0.56	0.73
R2	0.90	0.79	0.85	0.79	0.94	0.94	0.98	0.96	0.35	0.60
FTIR										
ME MJ/kg	All	Leaves	Stems	Coprosma	Griselinia	Hoheria	Mahoe	Pittosporum	Pseudopanax	Salix
n	171	86	85	35	27	28	10	35	16	20
Observed	10.21	11.08	9.34	10.60	10.66	9.75	8.07	10.01	11.24	10.19
Predicted	10.24	10.98	9.49	10.58	10.57	9.85	8.01	10.18	11.25	10.14
Bias	0.02	-0.10	0.15	-0.02	-0.09	0.10	-0.07	0.18	0.01	-0.05
P- value paired t-test	0.43	0.02	0.0002	0.70	0.44	0.10	0.63	0.004	0.86	0.44
MPE	0.65	0.15	0.01	0.84	0.67	0.32	0.79	0.06	0.93	0.66
RPE	0.004	0.002	0.0002	0.02	0.02	0.01	0.08	0.002	0.06	0.03
CCC	0.94	0.86	0.91	0.93	0.80	0.94	0.95	0.94	0.95	0.82
R2	0.89	0.76	0.84	0.87	0.67	0.89	0.90	0.91	0.91	0.69

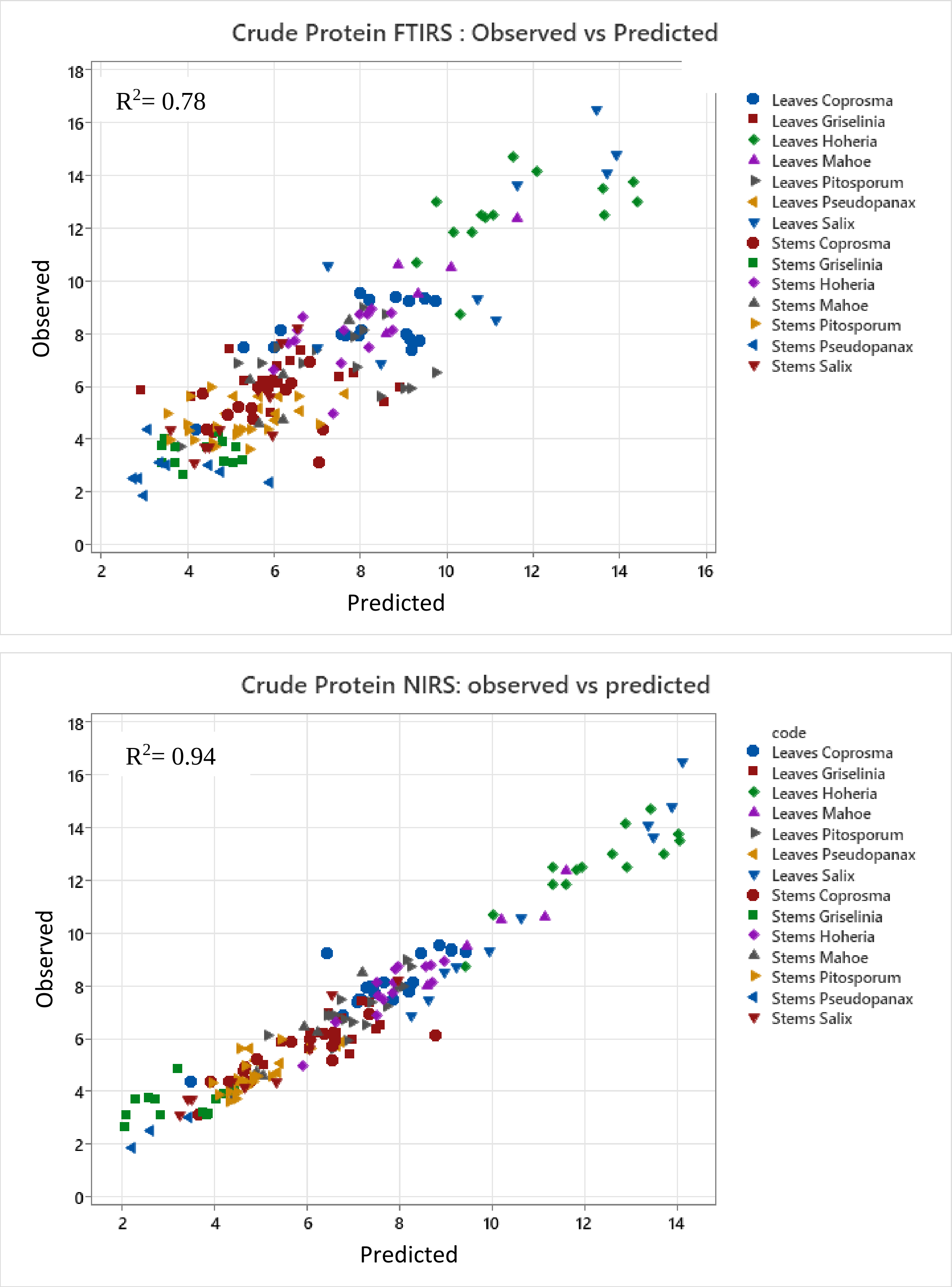
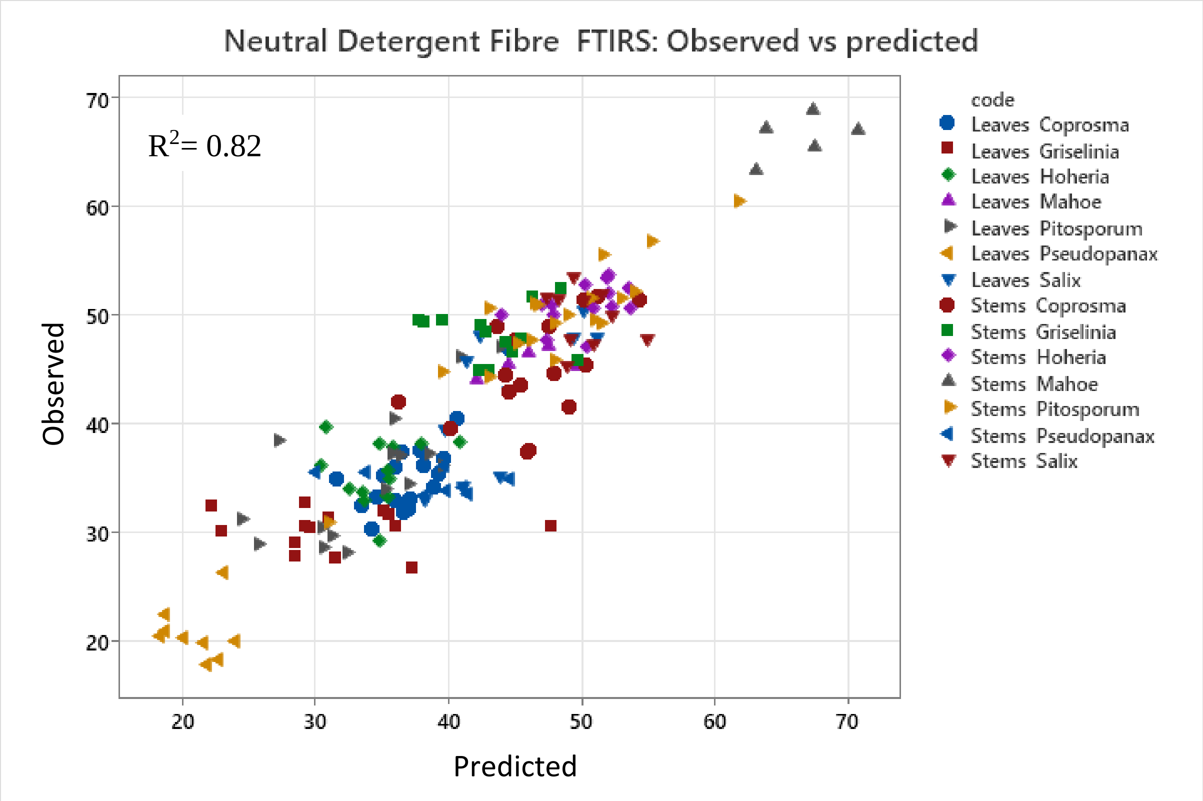


Figure 19: Plots showing observed vs predicted post prediction statistics R2 results for CP ATR-FTIR (top) and NIR (bottom).



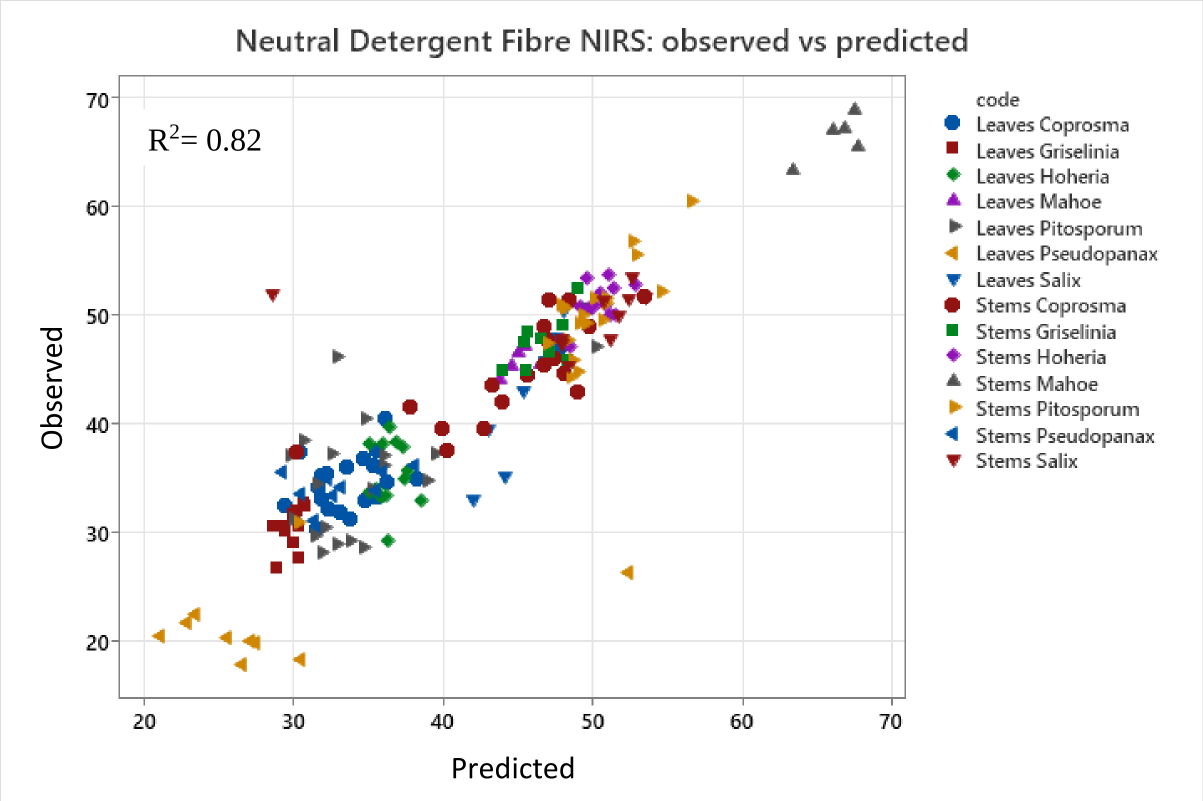
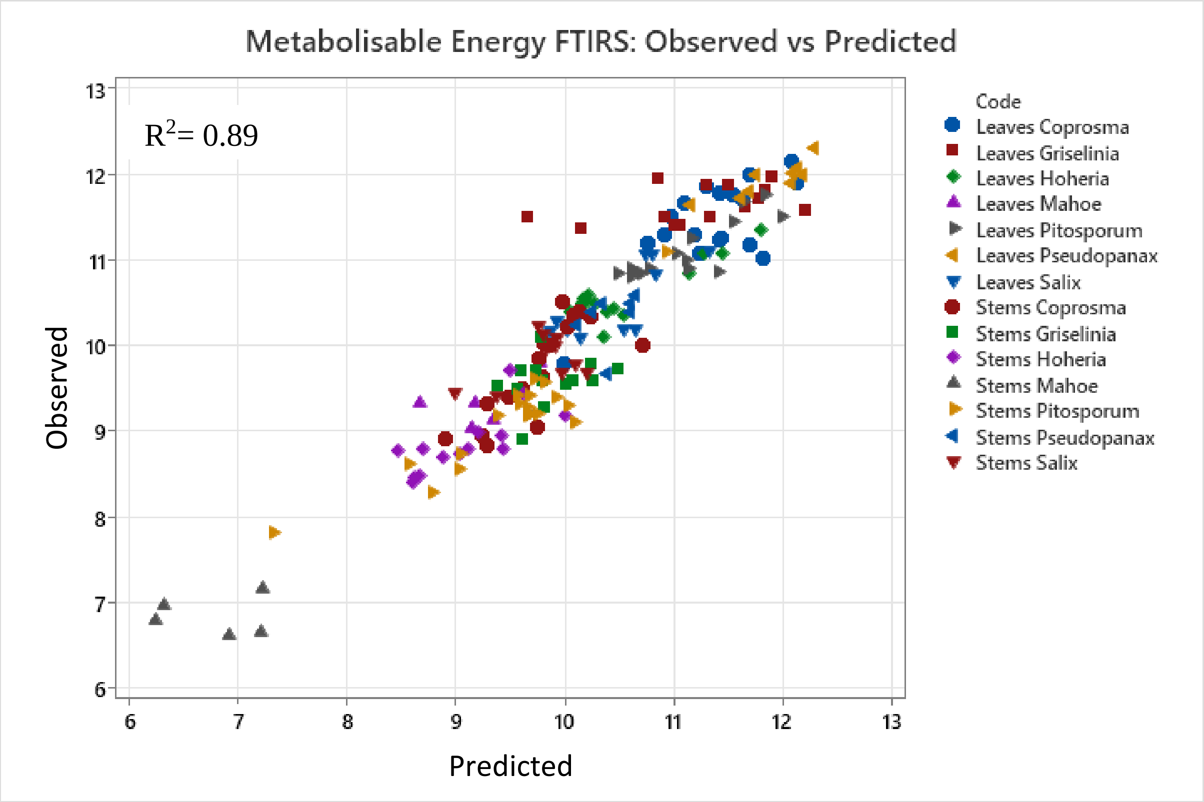


Figure 20: Plots showing observed vs predicted post prediction statistics R2 results for NDF ATR-FTIR (top) and NIR (bottom)



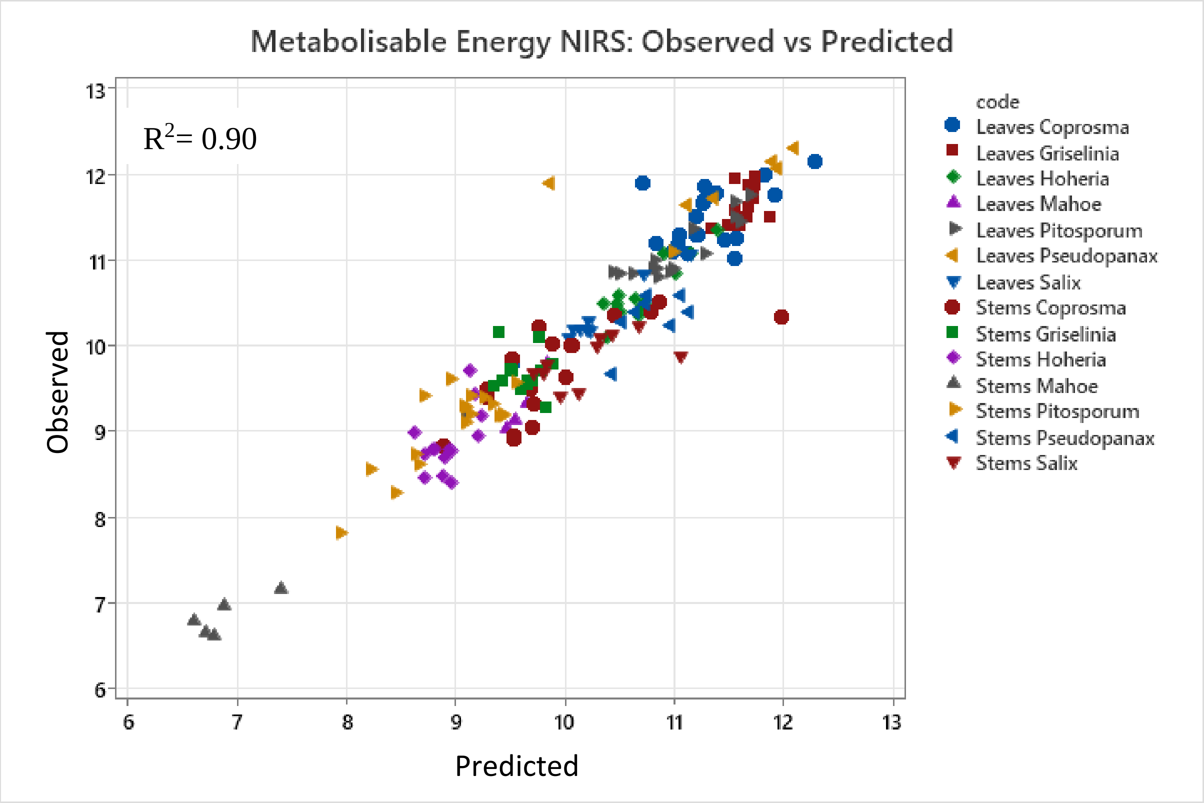


Figure 21: Plots showing observed vs predicted post prediction statistics R2 results for ME ATR-FTIR (top) and NIR (bottom)

Chapter 6.0: Discussion

This section will discuss the nutritional values predicted, regression results and performance of the prediction for all analytes, followed by the post prediction statistics focusing on three more nutritionally significant analytes, CP, ME and NDF. The sections will be separated by sets of results and further into separate analytes.

6.1 Nutritional Values

Native shrub species are becoming an increasingly popular alternative supplementary forage for sheep on steep hill country slopes in New Zealand (Norton, 2018). With this comes a need to determine the nutritional content values quickly, accurately and cheaply. Two prediction methods FTIR and NIR can do this however there are no excising calibrations for these native shrubs.

Very little research has been conducted on native shrubs that could be used as forage in New Zealand. As a result, there was a small number or no nutritional values for some species reported in the literature. This research study provides wet chemistry results to fill in some of these gaps and strengthen the current literature. In addition to this, nutritional values were successfully predicted for CP, ME, NDF, ADF, Ash and In vitro DOMD using NIR and FTIR spectroscopy and chemometrics. As there were no previous nutritional content predictions of native species for either method, these results are the first of their kind. This section will discuss the nutritional value results and how they compare and contribute to the literature. This study shared a subset of samples with Simmonds (2020) and Wangui (2023) so there was overlapping wet chemistry data.

6.1.1 Wet Chemistry

The nutritional values of native shrubs were extremely sparse across previous literature without the studies by Simmonds (2020) and Wangui (2023). The recent studies provided wet chemistry values of all analytes of interest for 5/6 native species, with the only exception being Mahoe. Possibly more significant is that they both used the same wet chemical methods to determine the nutritional content, allowing a quality and relevant comparison of values determined in this study. The similarities and differences between this study and current literature have been split into analytes and discussed. The arrangement of largest to smallest species for each analyte, has not been compared in this section, as it follows the same trends as the predicted values which have already been mentioned in the results section.

6.1.1.1 ADF

The literature regarding ADF for native shrubs was relatively good with at least two references to compare with for each species. Starting with the control Salix, this study 33.92% A, 29.50% L and 38.34% S, reported values that were similar to Simmonds (2020) 34.1% L, 40.0% S.

All of the native species had previous studies to compare to, with the majority agreeing with the values determined in this study. Coprosma had values of 29.61% A, 24.35% L and 35.45% S which overlapped very well with Simmonds (2020) 24.1% L and 35.5% S and Wangui (2023) 22.4% L and 36.7% L. Kurokawa et al, (2010) overlapped well with the all value 29.7%. Kurokawa et al. (2010) recorded leaf samples of Griselinia as 26.4%, slightly higher than 20.89% L found in this study. The two additional values 27.76% A and 38.62 S from this study show Kurokaawa’s value sits between the leaf and stem values. Values from Simmonds (2020) 21.2% L and 39.9% S, and Wangui (2023) 22.3% L and 38.6% S were similar to those in the present study.

The wet chemical results for Pittosporum, 30.53% A, 22.37% L and 38.70% S, were similar to those in Simmonds (2020) 23.3% L and slightly lower for stems 35.6% S. Wangui (2023) had a slightly lower leaf and higher stem value, 20.7% L and 41.0% S. The other literature available was presented in Wardle et al, (2009) with 28.9%. The value was similar to the Pittosporum all value (30.53%) and sits between the leaves and stems value. This was expected as the sample used was a stem and leaf mix, so this value makes sense and agrees with other literature and the present study.

Pseudopanax had significantly lower values compared to Pittosporum with 20.81% A, 15.36% L and 26.86% S. These values were very close to those of Simmonds (2020) 15.0% L and 27.9% S. Kurokawa et al, (2010) had a higher ADF leaves value 23.0% compared to this study and the value presented by Simmonds (2020). This could be due to the study using older dried leaves for analysis, which according to (Bentley, 1979; Escdero at al., 1992; Bargali and Singh, 1994; Oppong, 1998) age of a leaf increases the fibre within the leaf, increasing the NDF and ADF value from analysis. This is increased when dealing with more woody species such as Pseudopanax.

The results for Hoheria 29.85% A, 19.37% L and 40.33% S align extremely well with the values from Simmonds (2020), 19.4% L and 42.2% S and Wangui (2023), 20.3% L and 41.5% S. The ADF% results for Mahoe leaves 30.10% L, overlap well with Wardle et al. (2009) 34.2%, and Kurokawa et al. (2010) 30.0%. The other tissue types, 43.51% A and 56.93% L have no literature values available for comparison, providing new values to the literature.

6.1.1.2 Ash

Ash has not been widely explored for the native species in this study, with Simmonds (2020) and Wangui (2023) making up the majority of literature values. Mahoe has been investigated the least, with only Fitzgerald (1976) as a reference.

Salix, 4.56% A, 5.26% L and 3.85% S had good similarities to Simmonds (2020), 5.7% L and 4.1% S. Oppong (1998) had a range of 5.9-6.5% which is slightly higher but could be explained by more mature leaves, which elevates the nitrogen and ash content within the leaf (Bentley, 1979; Escdero at al., 1992; Bargali and Singh, 1994; Oppong, 1998). Kemp, Barry & Douglas (2003) had an unusually small value of 1.6% from their edible DM leaf-stem sample in comparison. They acknowledged that a similar willow species in their experiment, *Salix matsudanax alba,* had an Ash % of 6.4. They also had seasonal values of ‘willow’ ranging from March 3.3% to spring 7.6% which overlaps more with the values in this study, and more significantly suggested the season was significant for Ash content of Salix. With this in mind, it makes sense that the Salix Ash% values from this study which were collected in July (winter), were lower than those in Oppong (1998), which were collected in September (spring).

Coprosma, Griselinia and Pittosporum shared Simmonds (2020) and Wangui (2023) as their references for Ash values. The Ash values for Coprosma and Griselinia matched up closely with those in Simmonds (2020) and Wangui (2023), with the addition of All, 7.01% and 6.10% for Coprosma and Griselinia respectively. Pittosporum, 7.74% A, 7.66% L and 7.82% S, had Ash values slightly lower than those found in Simmonds (2020) 8.2% L and 8.6% S, but slightly above values in Wangui (2023) 6.5% L and 6.7% S.

Pseudopanax values 7.04% A, 6.42% L and 7.72% S, agreed strongly with the Ash values from Simmonds (2020) 6.3% L and 7.6% S. In contrast, Fitzgerald (1976) 5.7%, was slightly lower than both sets of values whilst comparing leaf values. As this leaf data was collected over a long period and throughout the seasons, the 5.7% is an average of high and low Ash% and not from one set timeframe unlike the other two studies possibly explaining the lower value.

The results for Hoheria, 10.6% A, 11.96% L and 9.24% S, agreed strongly to Simmonds (2020) 12.2% L and 9.7% S, Wangui (2023) 11.6% L and 9.0% S, as well as Sims (2018) 12.1% L.

Mahoe 10.8% A, 12.73% L and 8.88% S, only had one value to compare with, 9.7% from Fitzgerald (1976). This value was similar to all and stems but a bit lower than leaves. Again, with Ash being season dependent, it is not a surprise that the values are slightly different when comparing values to a study that recorded leaves across 3 years and many seasons. Nonetheless, all new values add to the library of values and become part of the literature, and challenge or add to what is known.

6.1.1.3 CP

Salix, 8.10% A, 11.09% L and 5.10% S, shared a similar stem value to Simmonds (2020), 8.8% L and 4.0% S, however the leaves value was higher in the present study. Kemp, Barry & Douglas, (2003), 7.1%, shared a similar value to Salix All and sat well between leaf and stems values of both this study and Simmonds (2020). As their study samples included edible leaves and stems down to <5mm, the value is reasonable and overlaps between the expected CP%.

Coprosma 6.71% A, 8.05% L and 5.23% S, Griselinia 4.97% A 6.31% L 3.63% S, Pittosporum 5.72% A 6.94% L 4.51% S and Hoheria 0.18% A, 12.52% L and 7.83% S, all had values very similar to those in Simmonds, (2020) 7.8% L and 4.5% S, 6.0% L and 3.4% S, and 6.6% L and 4.9% S, and those in Wangui (2023) 7.9% L and 4.7% S Coprosma, 6.2% L and 4.2% S Griselinia, 6.2% L and 4.2% S Pittosporum and 14.0% L and 8.3% S. The values in the present study are between Simmonds (2020) and Wangui (2023) for Hoheria.

Pseudopanax, 4.16% A, 5.35% L and 2.83% S, also had very similar CP values to Simmonds (2020) 5.4% L and 2.6% S, however the other reference did not share a similar value for leaves. Fitzgerald (1976) had a CP value of 8.6% for leaves, which was more than 3% above what this study and Simmonds (2020) determined. Something similar was seen with Mahoe, where Fitzgerald (1976) had a leaf value of 14.5%, 4.3% higher than the leaf value found in this study, 8.15% A, 10.2% L and 6.09% S. As the CP values given by Fitzgerald (1976) are higher than more recent literature and this study, the method of obtaining CP may have changed since 1976 resulting in a difference in values even for the same species. Unfortunately, the method was not given as the analyses was carried out by the analytical group DSIR's Applied Biochemistry Division.

If there had been more literature for these native species to compare with, even if the samples were taken in different seasons, the CP would be expected to remain similar throughout the year. This is mainly due to the species being evergreen, allowing the process of leaf senescence throughout the changing seasons creating an invariable CP (González-Zurdo, 2016). As very little literature for these species is available, further investigation will be required to understand the natural range of CP% of these native species.

6.1.1.4 ME

The Salix results in this study match up well to those given in Simmonds (2020) 10.5 MJ/kg L and 10.0 MJ/kg S compared with this study's 10.19 MJ/kg A, 10.53 MJ/kg L and 9.85 MJ/kg S. Kemp, Barry & Douglas (2003) had an ME of 9.7 MJ/kg which sits slightly lower than the stem value in Simmonds (2020) and this study. This is not a significant difference and since the sample used to obtain this value was made up of leaf and stem content, it is possible there was more stem content measured lowering the value.

Overall, the ME values for Coprosma, Griselinia, Pittosporum and Hoheria in this study, were similar with the literature values but slightly lower than Simmonds (2020) and Wangui (2023).

Pseudopanax, 11.2 MJ/kg A, 11.96 MJ/kg L and 10.36 MJ/kg S, had strong agreement in values with Simmonds (2020), 12.2 MJ/kg L and 10.8% S. In contrast, Fitzgerald (1976) jumps to a massive 21.6 MJ/kg which is completely different by a significant amount. A similar instance occurred with Mahoe, 8.07 MJ/kg A, 9.32 MJ/kg L and 6.38 MJ/kg S, where the only known values were from Fitzgerald (1976) and were far higher with a range of 17.8 MJ/kg -18.8 MJ/kg. Small variations are expected between literature values due to specific factors such as season, however the massive increase in Fitzgerald (1976) cannot be from this alone. The analyses of ME for Fitzgerald (1976) took place in 1969 and 1974, whilst the method used in the present study was proposed in 1977 by Roughan & Holland (1977). Therefore, the method used in Fitzgerald is outdated in comparison, which can explain the significant difference in results.

6.1.1.5 NDF

The NDF values found in the native shrubs overlap well with the available literature, of which Simmonds (2020) and Wangui (2023) are most prevalent.

The overlap of NDF values of Salix, 46.25% A, 42.58% L and 49.93% S, with Simmonds (2020), 48.7% L and 52.8% S, was similar for stems but lower for leaves. Oppong (1998) reported values of 35.7-38.7% (1 month - 6-month-old leaf), much lower than Simmonds (2020) and the present study. There are many possible explanations for why NDF is lower in Oppong (1998). NDF increases in periods of water stress, commonly occurring in summer when there is less rainfall and warmer temperatures (Moura et al, 2010). As this study and Simmonds (2020) collected samples during the summer and Oppong (1998) collected samples in spring this may be a reason for some difference in NDF. Furthermore, the Salix plants used for collection in Oppong (1998) were aged 3-4 years old compared to the limited 8 months in Simmonds (2020) and the present study. As a result, the more established root system of the older plants could cope with water stress more efficiently, therefore maintaining a lower NDF compared to a younger plant (Moura et al, 2010).

The NDF values for Coprosma, Griselinia, Pittosporum and Hoheria were for the majority, very similar to Simmonds (2020) and Wangui (2023), with only three slight differences. The leaves value for Coprosma was slightly higher in Wangui (2023) 37.4% compared to this study, 35.17% and Simmonds (2020) 34.7%. It was Simmonds (2020) that had a slightly lower NDF value in Pittosporum stems compared with the present study 49.5% and 52.0% in Wangui (2023). The last notable difference was in Hoheria 43.2% A, 35.43% L and 50.98% S, which had similar leaves to value to Simmonds (2020) 35.5% L and 53.1% S, but Wangui (2023) was slightly higher 37.6% L and 53.5% S.

Interestingly, the stem values from Wangui (2023) and Simmonds (2020) were very similar, but both higher than that in the present study. Pseudopanax 27.17% A, 20.80% L and 34.26% S had good similarity to its only reference values from Simmonds (2020) 21.0% L and 35.4% S. Mahoe had NDF values of 56.02% A, 45.69% L and 66.36% S, but there was no published literature for this to be compared to.

6.1.1.6 *In Vitro* DOMD

In Vitro DOMD is a less discussed nutritional component, as ME is more popular (DOMD*0.16 = ME). As a result, Simmonds (2020) and Wangui (2023) are the only values to compare with for these native species. Salix, 63.69% A, 65.82% L and 61.57% S, and Pseudopanax, 70.0% A, 74.75% L and 64.73% S, both only have one reference from Simmonds (2020) but show good overlap 63.7% L and 60.3% S and 73.8% L and 64.1% S respectively. The values from Simmonds for Coprosma, Griselinia, Pittosporum and Hoheria are very similar to those in this study. Although Wangui (2023) also has similar values for all these species, they are all shifted up a few percent higher. As this is seen across all of the species, it is likely a factor that impacts In Vitro DOMD like season. The values that Wangui (2023) used to compare stem and leaf nutritional content were from samples collected in spring (October), whereas the samples in this study were collected in winter (July). Wangui (2023) concluded there were differences between season for some nutritional content however he did not include In Vitro DOMD in that part of the research, only differences between tissue type (Wangui, 2023). As a result, it is probable that season affects *In Vitro* DOMD, but this will have to be investigated further for any firm conclusions to be made.

Mahoe reported values of 50.46% A, 58.23% L and 42.70% S. There is no current literature available to compare this with, so this result is valuable.

6.1.2 Predicted Values FTIR and NIR

This section discusses the differences between FTIR and NIR predicted values and the corresponding wet chemistry (Tables 19-21) for the whole panel of analytes. As there was no previous literature of predicted values, comparison can only be made to the wet chemistry literature. This section will only describe if the result was similar, higher, or lower than wet chemistry literature, as the wet chemistry was previously discussed above in depth. Overall, the majority of FTIR, NIR and wet chemical values aligned very closely with each other across tissue type and analyte.

6.1.2.1 ADF

ADF had one significant difference between FTIR, NIR and wet chemistry values. Pseudopanax stems had an unusually high FTIR value 31.79%, compared with NIR 26.52%, and wet chemistry 26.86%. The wet chemistry literature was 27.9% for stems, fitting between these values (Simmonds, 2020). All of the other values were very similar, with the most similar coming in Salix leaves, 29.61% (FTIR), 29.53% (NIR) and 29.50% (wet chemistry).

6.1.2.2 Ash, CP and ME

The predicted results for Ash, CP and ME for FTIR and NIR were similar to each other and the wet chemistry values. In addition, there were no patterns of under or over predicting present for FTIR and NIR when comparing to the wet chemistry value. Since FTIR and NIR were similar to the wet chemistry, the values predicted are similar to the wet chemistry within the literature also.

6.1.2.5 NDF

The prediction values were typically very similar between FTIR and NIR with the wet chemistry values. There were three results however that did not follow this.

The first difference is observed in Griselinia all, where both FTIR (37.34%) and NIR (37.59%) are underpredicting (-2.08% and -1.83%) compared to the wet chemistry value (39.42%). The predicted values in leaves and stems were similar to the wet chemistry values, with FTIR predicting slightly higher for leaves and lower for stems, and NIR predicting slightly higher for leaves and lower for leaves. If these values were averaged to make an 'all' prediction the values would be 39.58% FTIR and 39.60% NIR, which are very similar to that of the wet chemistry value 39.42%. Therefore, it is the all prediction for mixed leaf and stem values that causes a prediction lower than expected.

The second surprising result is seen in Pseudopanax all, where both FTIR (29.33%) and NIR (30.89%) predictions are higher (+2.16% and +3.72%) than the wet chemistry value (27.17%). Interestingly, the individual predictions for leaves and stems had very similar values between the FTIR, NIR and wet chemistry, Leaves: 20.64%, 20.41% and 20.8%, and Stems: 35.21%, 34.82% and 34.26% respectively. In figure 20, the NDF NIR plot showed one high predicted value for Pseudopanax leaves (yellow triangle) far away from the main cluster of samples. This lone sample may have contributed to a slightly higher prediction for the all tissue type and leaves, however this extreme value was not present in the FTIR plot and yet it also gave a high prediction for the all tissue type. Therefore, it is more likely to be an issue with the prediction for this specific tissue type and species.

The final unexpected result is seen in Mahoe stems in which the FTIR prediction (61.83%) is significantly lower than the NIR (66.36%) and wet chemistry (66.36%) values. The Mahoe FTIR predictions for the other tissue types all and leaves (55.26% and 44.06% respectively), were similar to NIR (55.74% and 44.82% respectively) and the wet chemistry values (56.74 and 45.69% respectively). The prediction method was run the same as the others, so it is strange this under prediction occurred in stems only. The way the calibration equation and prediction model were made to minimise the chance for an outlier to translate into the final prediction. Further analysis of the prediction results was carried out in the post prediction statistics and discussed further down the discussion.

6.1.2.6 In Vitro DOMD

Overall, there is a high agreement of values between FTIR and NIR predicted values and wet chemistry values. The largest disagreement comes in Hoheria leaves where the FTIR value (67.99%) is higher than the NIR (65.83%) and wet chemistry value (66.35%). FTIR value is 1.64% higher whilst NIR is 0.52% under the wet chemistry value. This difference is not that significant however, as these are much larger values when comparing them to CP, ME and Ash so percentage wise they are still very similar. For example, as a percentage the FTIR is only 2.5% larger and NIR is 0.78% smaller than the wet chemistry value. Therefore, the predicted values for In Vitro DOMD are comparable to the gold standard wet chemistry values.

To understand these unexpected prediction values better, more research and tests should be completed to determine why some predictions are better than others and what factors affect predictions of different analytes, species and tissue types. NDF had the most issues with predictions being slightly off compared with the wet chemistry values, where there were examples of both FTIR and NIR over and underpredicting values. There was no bias of either prediction method to over or under predict, but rather a mix changing between analyte, species and tissue type.

6.2 Partial Least Squares calibration and prediction for FTIR and NIR

The discussion will be broken down into analyte sections. Unlike the previous section, individual species are not discussed here. This discussion will focus on train/test prediction results; however, calibration cross-validation results are discussed where necessary for a full interpretation of specific results. All the R^2 results for calibration ('All Data' and 'Outliers Removed') across all analytes and tissue types were good, above 0.80, with most being above 0.90. These would all be considered high quality predictions, however if they don't also have a cross validation greater than 0.51, it could suggest overfitting of the calibration set. This is seen in a few cases where the 'training data set' had a very high R^2 but low CV, resulting in the 'test data set' R^2 (pred) being much lower. Interestingly, this was only observed for the FTIR method and discussed below.

As there were no previous prediction models for the native species used in this study, literature using similar MIR and NIR prediction models for different species are used to compare how R^2 and RPD performed for the same analyte. As R^2 only reflects the goodness of fit of the model to the data and high RPD indicates better predictive capability, the highest RPD will be the factor determining the best model for each analyte, reporting its matching R^2.

6.2.1 ADF

ADF produced good model predictions for both FTIR and NIR regarding R^2 and RPD. The training R^2 results were very high for FTIR and NIR however, the R^2 cross validation for FTIR stems was low, 0.52 but still considered ok for quality predictions (0.51<CV). The FTIR leaves CV was low 0.31, suggesting poor quality prediction. The prediction R^2 was considered good quality for FTIR and NIR all (0.71 and 0.78 respectively), NIR stems and FTIR leaves (0.77 and 0.75), excellent for NIR leaves (0.92), and moderate FTIR stems (0.53). The RPD was higher for NIR than FTIR for all tissue types. NIR had the highest RPD 3.36 in stems suggesting excellent prediction, and a high-quality prediction for all and leaves (2.80 and 2.82). FTIR had RPD above 2 for all tissue types also suggesting quality prediction, with all being largest (2.52) and stems the smallest (2.23). The ability of the model to predict with new spectra is indicated by RMSECV. The RMSECV was relatively low for most values however it was largest for NIR all (9.91). This can indicate that this particular model may not have as high quality R^2 and RPD as the current data suggests. Overall, the FTIR showed higher RMSECV with the exception of NIR in all. The MSE(pred) is also higher for FTIR, suggesting a slightly less robust model when comparing it to NIR.

Several studies have developed prediction models of various species for ADF using MIR or NIR spectroscopy, showing mixed results and model performance. The best results in this study for ADF (0.75, 2.44 FTIR L, 0.77, 3.36 NIR S) show a greater performance for

R^2 and RPD than Cleland et al, (2018) (0.70, 1.79) FTIR and Asekova et al, (2016) (0.5, 1.97) NIR. The R^2 in Cleland et al, (2018) is not much different to this study, but the difference in RPD is significant as it reflects the model in the current study was able to predict values more accurately. This study showed significant improvement of R^2 and RPD compared with Asekova et al, (2016) for ADF prediction. A study with similar NIR performance with good R^2 and excellent 3<RPD was by Yang et al, (2017) using a prediction on Italian ryegrass (0.89, 3.1). Monrroy et al, (2017) outperformed these studies using a prediction on soybean samples resulting in both excellent R^2 and RPD (0.90, 5.0). Richardson and Reeves (2005) compared FTIR and NIR R^2 and found MIR had slightly higher R^2 than NIR for ADF (0.91, 0.89), however there was not enough evidence to suggest one was better than the other. Richardson and Reeves (2005) suggested the possibility of MIR model having a slight overfitting issue boosting R^2 whilst also increasing its error. NIR had a lower R^2 but also lower error, leading them to question whether the NIR model was more robust than the FTIR model.

6.2.2 Ash

Both FTIR and NIR methods produced high quality Ash prediction R^2 and RPD results for leaves, 0.73, 3.00 and 0.90, 3.96 respectively. The all and stems groups were less positive for FTIR, with a moderate and poor R^2(pred), 0.55 and 0.33. The CV result for all was low 0.52, just above the minimum value considered for quality prediction. The result for stems was below this, 0.22, which then becomes no surprise as to why the model performed so poorly. The NIR had good R^2(pred) for all and stems (0.86 and 0.79). RPD was higher for NIR than FTIR with excellent values for all and leaves for NIR whereas FTIR only had leaves as excellent. The RPD for stems was comparably low in NIR, only

2.24 however this could be explained by the lower training R^2CV 0.63, suggesting the stems model may be less robust than the model used for all and leaves. The RPD was above 2 for all and stems, meaning even with the low R^2, the prediction models could still be useful for nutritional content prediction. Monrroy et al, (2017) had similar findings where a low R^2 was not always representative of a good model and the RPD was the more significant metric to measure model prediction ability. The MSE was overall low across both methods, with NIR being lower than FTIR across the different tissue types. RMSECV shared this same trend, with FTIR and NIR stems values, 0.93 and 0.87 being the most similar. The low RMSECV suggests slightly better model quality in NIR for ash, but the difference here isn't that great.

There were few predictions of Ash within the literature for NIR or FTIR to compare with. Hell et al, (2016) compared wheat bran predictions for MIR and NIR with great R^2 results: 0.85 and 0.91 respectively. This was higher than the current studies results, 0.73, 3.00 FTIR and 0.90, 3.96. NIR.

6.2.3 CP

The calibration and prediction results were polarising between methods with FTIR producing moderate and poor R^2 prediction and RPD just above 2. NIR on the other hand produced excellent predictions for both R^2 and RPD across the three tissue types. CP was the worst prediction results for FTIR, whilst being the best for NIR.

The very first regression model for FTIR in 'All Data' saw excellent R^2(calib) results however R^2CV had two poor values for stems and leaves (0.30 and 0.32) and a moderate prediction for all, 0.54. These high R^2(calib) and low CV values continued into the 'Training Data set' but here the all R^2CV had also dropped into the poor range (CV<0.50).

This showed evidence for overfitting of the model which resulted in a drastic drop for R^2(pred) with a moderate prediction for all and stems, 0.57 and 0.52, and poor prediction for leaves, 0.20. In comparison, NIR R^2 and R^2CV results were excellent throughout calibration and prediction resulting in R^2(pred) of 0.91 A, 0.93 S and 0.95 L. The RPD for FTIR was barely above 2 across the tissue types with the best in all, 2.12 A, 2.03 S and 2.06 L, still suggesting high quality predictions. NIR produced excellent RPD well above 2, 4.12 A, 3.79 S and 5.58 L. RMSECV of FTIR was around double as large as NIR.

There were many previous prediction models for CP for a range of species with the majority using NIR, and two studies comparing FTIR and NIR predictions. In the following section, best CP results from this study, 0.57, 3.00 FTIR and 0.95, 5.58 NIR will be compared with the available literature.

Cleland et al, (2018) achieved a good R^2 and excellent RPD value from a MIR prediction of CP (0.72, 2.33). This was higher than the prediction results in this study for FTIR.

The NIR results in the current study had similar R^2 and higher RPD than Yang et al, (2017) (0.91, 3.2) and Asekova et al, (2016) (0.91, 3.25). The R^2 was much lower in Berzaghi et al, (2005) (0.71), and Monrroy et al, (2017) (0.53, 3.5). Hell et al, (2016) compared the prediction of CP for MIR and NIR and found MIR had a higher R^2 than NIR, 0.75 and 0.66 respectively. This was however the only analyte in their study to be higher for MIR than NIR.

Hell et al, (2016) suggested that due to the protein absorption bands having characteristic bands spread across the MIR spectrum, the multivariate analysis would be better able to select these bands used for prediction better, compared to the NIR bands which are less separated. With further analysis, Monrroy et al, (2017) determined that the NIR was more robust than MIR for a one size fits all model, due to higher error in MIR from the characteristic bands during multivariate calibration. Shi et al, (2019) was the other study comparing MIR and NIR predictions of CP, with better results for MIR and NIR than the present study. They achieved excellent R^2 and RPD for both MIR (0.96, 4.9) and NIR (0.98, 8.0), concluding NIR has a slight superior prediction over MIR at this stage.

6.2.4 ME

The calibration and prediction of ME was high quality for both FTIR and NIR methods. All of the training set R^2 results were excellent and similar between FTIR and NIR. R^2(pred) was in the good range for FTIR with the smallest in all 0.76 and largest in leaves, 0.83. NIR had higher R^2(pred) than FTIR with two good and one excellent result ranging from 0.83 in all to 0.91 in leaves. There was no issue with low R^2CV unlike a few other analytes, suggesting no overfitting with most values being excellent ($0.74<R^2$CV), and FTIR stems and leaves being 0.62, in the range for useful quality prediction. The RPD was excellent, 3 or above for both methods and all tissue types, with the highest as 3.52 for FTIR stems and 3.89 for NIR leaves. The MSE and RMSECV are very small and similar between FTIR and NIR.

Lobos et al, (2013) used FT-NIR spectroscopy with PLS regression to predict nutritional quality of pasture from Chile. The prediction of ME was of high quality with R^2 and RPD 0.94 and 4.4. This result is slightly higher than the present study. Cleland et al, (2018) reported FTIR ME prediction results slightly lower than that in the present study for R^2 and RPD, 0.72 and 2.33.

6.2.5 NDF

The prediction model resulted in high quality results for NIR and moderate results for FTIR. The R^2 for calibration and the training sets were all good or excellent for FTIR and NIR. The R^2 CV results for the calibration of FTIR stems, leaves and NIR leaves ranged from 0.55-0.58 putting them into the lower end of useful for quality prediction. The training data set saw the FTIR R^2 CV drop below 0.5, to 0.24 and 0.47 for stems and leaves, making the predictions poor and suggest overfitting of the model. The R^2 prediction partially reflected this with moderate values for FTIR all and stems, 0.56 and 0.50, but an unexpected, good value for leaves 0.84. NIR had good R^2 prediction for all and leaves, and excellent in stems. The RPD was considered excellent across the tissue types with the highest in stems for both methods (2.54 FTIR and 3.4 NIR). The RPD was surprisingly similar between FTIR and NIR even with the notable difference in R^2, with NIR having slightly larger values for all (2.33 and 2.37) and leaves (2.28 and 2.31). Stems had a large difference between RPD with 2.54 for FTIR and 3.4 for NIR. The MSE(pred) error was especially large in all for both methods compared to stems and leaves. This was partially due to the separation in stem and leaf samples the model must predict, causing more variation and thus a larger base error. A larger error was also found for NDF in Cleland et al, (2018) compared to the other analytes. The other obvious suggestion of high error is that the model is overfitting.

There were several predictions of NDF using NIR, one using MIR and one study comparing the prediction of both FTIR and NIR in the literature. Richardson and Reeves (2005) compared FTIR and NIR R^2 prediction determining there was no significant difference between methods for prediction accuracy and model robustness. The single reported MIR study was conducted by Cleland et al, (2018) and reported good R^2 higher than the present study, and excellent RPD similar to the present study, 0.86 and 2.6. The best NIR result in stems (0.90, 3.40) has a higher quality prediction than all of the current literature NDF R^2 and RPD predictions such as Yang et al, (2017) 0.88, 3.0, Monrroy et al, (2017) 0.86, 3.4, Asekova et al, (2016) 0.77, 2.07 and Berzaghi et al (2005) 0.82. The all and leaves NIR results are far more similar to Asekova et al, (2016).

6.2.6 *In Vitro* DOMD

The FTIR and NIR calibration results were excellent for *In Vitro* DOMD, producing high quality predictions and robust models. The training set R^2 were excellent for all tissue types with the highest in stems for FTIR and NIR. The R^2CV was also very good above 0.7 for all results. The R^2 prediction gave good results for NIR and FTIR all and stems but dropped to moderate for leaves. NIR had the higher prediction value but for stems R^2 prediction was higher in FTIR than NIR, 0.82 and 0.77 respectively. This was strange because NIR stems had the highest R^2 and R^2CV values, so it would've made sense to have the highest R^2 prediction also. The same scenario occurred in ME, so it was not only confined to this prediction model but something more complex at play. What's also interesting is that the RPD is considered above excellent (3<RPD) in both even with the low R^2, suggesting the model had poor fit with the data but the model itself was still able to predict high quality nutritional content values. Without further investigation and testing it is hard to speculate why this only occurred in ME and *In Vitro* DOMD for NIR stems, as a similar result has not been discussed in any literature. The RPD was above 3 for all tissue types except for FTIR leaves which was 2.47, still considered excellent. The highest RPD was in stems for both FTIR (3.33) and NIR (3.60). The best results were 0.82 and 3.33 for FTIR, and 0.77 and 3.60 for NIR stems.

The literature regarding *In Vitro* DOMD predictions is sparse. Cleland et al, (2018) used MIR to predict *In Vitro* DOMD of temperate forage feed gaining moderate success for R^2 and RPD, 0.72 and 1.62. The present study prediction results were slightly higher for R^2 but significantly improved for RPD, suggesting the method used produced a more accurate and robust prediction model. Duranovich et al, (2020) used a NIR method for the prediction of *In Vitro* DOMD for a ryegrass-white clover mixed herbage for dairy farms, with excellent results for R^2 and RPD, 0.95 and 4.55, outperforming the current study prediction.

6.4 Post Prediction Statistics

The post prediction statistics provided a different approach to determine how well the FTIR and NIR prediction models performed for CP, ME and NDF. This did not only include the three tissue types all, leaves, and stems but also all of the individual species. The goal for this test was to determine if one blanket model was adequate for quality predictions or if individual models were needed for each species. Furthermore, these outcomes were compared between NIR and FTIR methods. The R^2 of the post prediction statistics do not match the R^2 PLSR prediction (test) as that was based off a small test set whereas the post prediction results are based on the full outliers removed data set.

The following sections are separated by analyte, discussing the results from Tables 19-21 and figures 19-21. Bias, MPE and RPE were found to be all relatively low and similar between FTIR and NIR for each analyte, so these are not discussed in depth. P-value paired t-test was used to test if the observed and predicted mean values were or were not significantly different from each other. The main focus on whether a model was useful was determined using CCC and R^2. The prediction results suggest that NIR is slightly better for the prediction of CP and NDF and equal to FTIR for ME prediction. This is based on the all tissue type model, which outperformed stems and leaves.

6.4.1 Post prediction CP

There was a clear difference between NIR and FTIR result in quality for CP prediction, with NIR performing better for almost all of the results for each metric tested. Not only was there very minimal bias in NIR, but the observed and predicted values were found to be not significantly different $0.05<p$ in the p value paired t test, for every result. FTIR on the other hand had a larger bias range and three results, stems, Hoheria and Pseudopanax with p values smaller than 0.05, meaning there was a significant difference between the mean observed and predicted values. MPE and RPE were about double the size for FTIR (1.39 and 0.21) compared to NIR (0.70 and 0.1), suggesting higher predictive accuracy from the NIR method. A study by Ferreira et al, (2014) saw that the protein of soybeans was predicted with high quality models for both NIR and MIR, 0.88 and 0.91 respectively. The study did a post prediction analysis and determined the relative error of prediction between their measured and predicted values was also higher for MIR (3.55) than NIR (3.04), agreeing with the results in the present study.

The CCC was excellent for all NIR results ($0.81<\text{CCC}$), considered perfect predictions with high reproducibility of measurements. FTIR only had half of its results considered excellent including all, leaves, Hoheria, Mahoe and Salix. The others were significant apart from Griselinia which dropped a level lower to moderate prediction and reproducibility. The all group in NIR was equal highest CCC at 0.97 with Hoheria, Mahoe and Salix. The FTIR all group was third highest (0.87) just less than Mahoe and Salix. The high CCC was a good indicator of high R^2, with these same groups being the highest.

In both NIR and FTIR, the all group (0.94 and0.78) was lower than Mahoe (0.95 and 0.89) and Salix (0.96 and 0.85), but above all of the other species and tissue types, agreeing that the all groups prediction is high for both methods. The quality of prediction was higher for NIR across all 10 groups having either excellent or good with the lowest R^2 0.81 for Coprosma. FTIR R^2 values were mixed, with no excellent values five good predictions, three moderate and two poor results for Griselinia and Pittosporum. The Griselinia low R^2 was somewhat expected due to moderate CCC, but Pittosporum, Pseudopanx, stems and Coprosma had significant CCC and yet still gave only moderate and poor R^2. The difference between NIR and FTIR may suggest NIR has a more robust prediction for CP that is less affected by low sample size (Ali, 1987). Looking back to the prediction results, CP was the worst FTIR prediction of any analyte, so, unsurprisingly, some of the R^2 for individual small sample size species ended up with poor results. Furthermore, CP was NIRs highest prediction result making the post prediction statistics excellent, strengthening the predictive ability of NIR. The all group does not have the best individual results for either NIR of FTIR however, as the individual species results are lower for the majority, it supports the idea of having one blanket prediction for all of the native shrub species having advantages over individual predictions. The CP plots in figure 19 show the all group R^2 for FTIR and NIR.

6.4.2 Post prediction ME

The post prediction results for ME were similar between the NIR and FTIR methods, with all and stems sharing almost identical results, whereas leaves favoured NIR slightly. Bias, MPE and RPE were very similar between methods across all of the different groups, neither showing superiority over the other. The p value paired t test results also showed similarities between methods with leaves, stems and Pittosporum having a $p<0.05$

meaning the observed and predicted mean values were significantly different from each other for FTIR and NIR. NIR had one additional species, Salix which also had $p<0.05$.

Continuing the similarities between NIR and FTIR, all but three CCC results were above 0.81 and considered perfect predictions with high reproducibility of measurements. Two of these CCC values were in NIR, Psudopanax moderate 0.56, and Salix significant 0.73. Griselinia was the species in FTIR that was not excellent but still significant 0.80. It is important to notice that Griselina was again lowest for FTIR CCC.

The R^2 results were slightly better for NIR all and stems (0.90 and 0.85) compared to FTIR (0.89 and 0.84) but this is not a significant difference to confidently say one is more superior to the other. NIR leaves was slightly better than FTIR, 0.79 and 0.76 respectively. The NIR prediction had higher R^2 for leaves, Hoheria, Mahoe and Pittosporum however FTIR still had excellent and good prediction. Griselina is excellent for NIR but drops to moderate for FTIR. FTIR had higher R^2 for Coprosma, Pseudopanax and Salix. The most significant difference was in Pseudopanax and Salix NIR R^2 0.35 and 0.60 compared to FTIR values 0.91 and 0.69. The low values in NIR were not a surprise after looking at the low CCC results suggesting they would not be as highly predicted or robust as the rest of the predictions. As both methods predicted some species better and worse than the other, and some R^2 as low as moderate and poor, the use of general tissue models over individual species models would be enough for quality prediction. As the all group is better than leaves and stems, this is therefore the only model that would be required for quality prediction of ME for native shrub species. Comparing FTIR and NIR plots in figure 21 the predictions look very similar with a few slight differences. The red squares belonging to Griselinia leaves do not fit well to the model and it is Griselinia that has the lowest R^2 for FTIR.

6.4.3 Post prediction NDF

The post prediction statistic results showed that neither NIR nor FTIR was completely superior to the other. Both prediction methods had mostly high-quality predictions, however some lower predictions were also present. NDF had a higher bias in both methods compared to the other two analytes. Griselinia was the only group to have $p<0.05$ for NIR, whilst FTIR had two, Coprosma and Pittosporum meaning these three species had observed and predicted mean values that were significantly different from each other. The MPE and RPE were relatively low for NIR and FTIR however the individual species had the highest error. The highest MPE and RPE were leaves, Pseudopanax and Salix for NIR and Coprosma and Pseudopaanx for FTIR. NIR had lower results for CCC with three results under 0.81, leaves Pseudopanax and Salix, compared with only two for FTIR, Griselinia and Salix. Between all, leaves and stems, all had the highest CCC and was identical between FTIR and NIR as 0.91. The R^2 mirrored the CCC results with the highest value in FTIR and NIR found as Mahoe, 0.95 and 0.99 respectively. It is also true for the lowest values found in Pseudopanx and Salix (0.23 and 0.10) for NIR and Griselinia and Salix (0.53 and 0.55) for FTIR. This showed that although NIR produced the highest R^2, it also produced the two worst results far under poor ($R^2<0.5$) whereas FTIR still managed to produce moderate prediction at worst. Similar to the other two analytes, it seems the low sample size makes for significantly more varied R^2 and less robust predictions compared with using the larger sample size all, leaves and stems groups. The all group was the highest of the three tissue types for FTIR and equal with stems in NIR, sharing the same value between methods, 0.82. As a result, it can be inferred that a single model is sufficient for quality prediction of NDF and the prediction methods are equally as good between FTIR and NIR using a single model.

Using a single leaves and stems model, makes more sense than individual leaves and stems, due to the context of wanting the nutritional content in the first place. Knowing the nutritional content is important when considering a sheep's nutritive requirements that need to be met by these native shrub species. It is known that sheep browse on leaves and stems up to 5mm in diameter (Kemp, 2003; Oppong, 2001; Wangui, 2023). Therefore, having a model which can predict the nutritional content of what a sheep is actually eating is potentially more beneficial for application, than two individual models for leaves and stems. Saying this, this study has produced results for all three tissue types, showing the potential of the prediction quality of a mixed tissue and individual predictions to enhance the literature.

6.5 Limitations of the study

This study had two main limitations. The first was the sample size of individual species. This limited the regression calibration and prediction model to low train/test groups which meant low CV and high prediction variability, leaning heavily on the training data. As a result, a blanket prediction of all native shrub species was used instead, showing high prediction quality for both methods.

The second was spectra quality for the FTIR spectra. Due to inexperience, when initially using the ATR-FTIR benchtop machine a background was recorded every 30-40 minutes. This was usually acceptable, however there were times the laboratory conditions changed (air conditioning flicking on/off and people coming in/out of the lab frequently) that was significant enough to impact the background, requiring a new background sooner than 30-40 minutes. As a result, several measurements were recorded with inaccurate backgrounds, potentially leading to some poor spectra quality, negatively impacting the prediction results. There are available pre-processing using multiplicative signal correction that normalise noise with PLS packages that can deal with these issues (Wehrens & Mevik, 2007; Innes et al, 2019; Liberda et al, 2021), but they were not employed in this study.

A trend that was observed in ADF, Ash, CP and NDF for FTIR was the presence of low cross validation performance for some predictions, much lower than NIR. This was also found in previous studies where it was determined that MIR spectroscopy had a higher sensitivity to the homogeneity and particle size of the sample, resulting in lower performance (Brás et al., 2005; Hell et al, 2016; Shi et al, 2019). As FTIR only requires a tiny amount per spectra, taking samples from the same place in the bag could impact the results. If one replicate sample was taken from the bottom of the bag (where fine particle size congregates) and one from the top of the bag (where larger particle size sit) the particle size could result in slightly different prediction results. Therefore, it is suggested that the sampling technique is consistent across all sample bags to avoid this potential issue. NIR used a handheld probe with a large lens which captured a range of particle sizes (Santos et al, 2013). FTIR has handheld probes, so it may be interesting to see if this would minimise this issue.

Chapter 7.0: Conclusion and Future Work

7.1 Conclusion

The results from this study have been able to address several questions and fill substantial gaps within the literature. Several key analytes were predicted, FTIR and NIR prediction models were evaluated and compared between FTIR and NIR prediction methods. Prediction values were successfully obtained for FTIR and NIR and compared to wet chemistry values, finding much agreement between methods as well as values within the literature.

High quality and robust prediction models were achieved for most analytes using both FTIR and NIR prediction methods. The highest NIR model was found in CP giving R^2 0.95 and RPD 5.58. The highest FTIR model was found in ME giving R^2 0.79 and RPD 3.52. Overall NIR showed higher predictive ability and slightly superior robustness compared to the FTIR models for all analytes, but FTIR still maintained excellent RPD>2. FTIR had its weakest prediction in CP, with the R^2 0.57 having moderate fit and RPD 2.12 suggesting high quality prediction. The lowest NIR result was in ADF, R^2 0.77 and RPD 3.36. Both FTIR and NIR prediction models performed well with significant predictive ability, comparable and in some cases better than literature predictions of other species. Post prediction statistics successfully evaluated the prediction of tissue types and individual species. The findings concluded that one blanket model was sufficient for quality CP, ME and NDF predictions of native shrubs. Furthermore, it also found the post prediction models for ME and NDF to be similar between FTIR and NIR with neither showing a significant advantage over the other, unlike in CP where NIR was superior to FTIR. In conclusion, this thesis has demonstrated the ability of FTIR as an alternative to

NIR as a fast, accurate and cheap prediction method to be used to analyse ME, CP, NDF, ADF, Ash and *In Vitro* DOMD for native shrub species.

7.2 Future work

It would be beneficial to investigate the calibration and prediction of individual shrub species. To achieve this, a larger sample set will be required to ensure there are enough samples for a reasonable train/test split and external validation sets that were lacking in this study.

In addition, the nutritional content analysis could be expanded to feature more components such as starch, sugars and sought after minerals. A step further than this could be to predict plant characteristics that are related to nutritional components such as digestibility and palatability of a plant.

As an ATR-FTIR benchtop spectrometer was compared with an NIR handheld probe, it would be of value to compare the NIR results with handheld FTIR prediction results. Would the handheld probe be able to improve on the benchtop prediction results and will these predictions compare to the NIR method?

A benefit of IR predictions is that they are relatively fast compared to wet chemistry. After a calibration model has been made, new samples will have to be freeze dried before analysis which takes time. A potentially faster alternative analysis would be to use fresh samples in a liquid phase, but this requires more testing. FTIR is used in applications such as milk nutritional content testing, therefore could potentially be used to determine nutritional content predictions from fresh samples using a liquid phase faster than the freeze-dried method.

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9.0 Appendix

Appendix 1: Wet chemistry results for native forage samples.

NutLab ID	Species	Tissue	DM %	Ash %	CP %	NDF %	ADF %	In vitro DOMD %	ME
TN20-102-01	Salix	Leaves	96.1	5.7	8.6	43.1	30.0	63.7	10.2
TN20-102-02	Salix	Stems	95.2	4.1	4.2	47.9	35.5	59.2	9.5
TN20-102-03	Pittosporum	Leaves	95.9	6.8	6.1	37.3	19.4	67.9	10.9
TN20-102-04	Pittosporum	Stems	94.7	7.1	4.3	49.2	36.5	58.3	9.3
TN20-102-05	Hoheria	Leaves	96.0	11.3	10.7	38.4	18.3	65.9	10.5
TN20-102-06	Hoheria	Stems	94.3	9.0	6.6	52.9	41.5	54.6	8.7
TN20-102-07	Pseudopanax	Leaves	96.6	6.4	5.1	20.2	14.2	73.2	11.7
TN20-102-08	Pseudopanax	Stems	94.0	7.8	2.8	33.4	28.3	64.1	10.3
TN20-102-09	Coprosma	Leaves	96.2	6.7	7.9	36.3	22.6	71.9	11.5
TN20-102-10	Coprosma	Stems	94.9	6.9	5.3	42.1	32.0	62.5	10.0
TN20-102-11	Griselinia	Leaves	95.9	5.1	5.1	30.6	19.2	71.9	11.5
TN20-102-12	Griselinia	Stems	95.0	3.6	2.7	52.5	43.6	55.8	8.9
TN20-102-13	Griselinia	Leaves	96.8	7.3	5.9	26.9	17.1	71.0	11.4
TN20-102-14	Griselinia	Stems	95.3	5.9	3.8	45.0	34.8	59.4	9.5
TN20-102-15	Pseudopanax	Leaves	94.9	5.7	5.2	17.8	13.0	72.8	11.6
TN20-102-16	Pseudopanax	Stems	91.9	5.6	2.4	36.2	28.9	60.5	9.7
TN20-208-01	Salix	Leaves	97.9	5.2	9.4	48.2	35.0	63.5	10.2
TN20-208-02	Salix	Leaves	97.3	5.7	6.9	50.5	33.7	63.6	10.2
TN20-208-03	Salix	Leaves	96.8	5.2	7.5	48.0	33.6	63.4	10.1
TN20-208-04	Salix	Leaves	97.4	5.2	8.8	48.0	34.1	64.1	10.3
TN20-208-05	Salix	Leaves	97.3	6.3	10.6	45.9	32.2	64.0	10.2
TN20-208-06	Salix	Stems	97.9	4.4	4.4	51.6	39.3	61.3	9.8
TN20-208-07	Salix	Stems	98.0	3.6	3.1	51.7	37.1	60.5	9.7
TN20-208-08	Salix	Stems	97.2	3.8	3.8	51.5	38.9	62.7	10.0
TN20-208-09	Salix	Stems	97.5	3.4	3.8	53.6	41.5	60.7	9.7
TN20-208-10	Salix	Stems	98.2	4.8	4.4	52.1	41.2	61.7	9.9
TN20-208-11	Pseudopanax	Leaves	98.1	6.4	5.6	26.3	16.5	74.4	11.9
TN20-208-12	Pseudopanax	Leaves	97.8	5.8	5.6	20.0	14.9	74.0	11.8
TN20-208-13	Pseudopanax	Leaves	98.2	6.2	5.0	18.3	14.0	74.7	12.0
TN20-208-14	Pseudopanax	Leaves	97.8	5.9	5.0	19.9	15.0	74.9	12.0
TN20-208-15	Pseudopanax	Stems	97.3	9.2	3.1	31.1	23.3	66.1	10.6
TN20-208-16	Pseudopanax	Stems	97.4	7.0	2.5	33.5	26.3	65.9	10.5
TN20-208-17	Pseudopanax	Stems	97.6	7.1	1.9	34.2	26.1	66.2	10.6
TN20-208-18	Pseudopanax	Stems	97.4	7.1	2.5	34.9	27.4	65.5	10.5
TN20-208-19	Griselinia	Leaves	98.0	7.0	5.6	31.4	21.5	71.8	11.5
TN20-208-20	Griselinia	Leaves	98.1	6.8	6.3	27.8	21.5	71.5	11.4
TN20-208-21	Griselinia	Leaves	97.8	7.3	6.3	31.8	22.8	71.2	11.4
TN20-208-22	Griselinia	Leaves	98.0	7.0	6.3	30.7	21.7	71.8	11.5
TN20-208-23	Griselinia	Stems	98.0	5.1	3.1	44.9	35.9	61.2	9.8
TN20-208-24	Griselinia	Stems	98.0	4.7	3.1	47.6	38.4	60.3	9.6
TN20-208-25	Griselinia	Stems	98.3	4.9	3.8	49.1	39.2	60.1	9.6

Appendix 1: Wet chemistry results for native forage samples (continued).

NutLab ID	Species	Tissue	DM %	Ash %	CP %	NDF %	ADF %	In vitro DOMD %	ME
TN20-208-26	Griselinia	Stems	96.9	4.9	3.8	48.5	39.7	59.9	9.6
TN20-208-27	Coprosma	Leaves	97.5	7.3	7.5	31.9	23.1	70.4	11.3
TN20-208-28	Coprosma	Leaves	97.9	7.3	8.1	35.2	24.3	69.7	11.2
TN20-208-29	Coprosma	Leaves	97.3	6.8	7.5	33.0	23.9	70.3	11.3
TN20-208-30	Coprosma	Leaves	96.9	7.1	6.9	34.6	24.1	69.6	11.1
TN20-208-31	Coprosma	Leaves	97.7	7.2	7.5	31.2	22.2	70.8	11.3
TN20-208-32	Coprosma	Leaves	96.8	5.6	4.4	47.0	36.3	61.2	9.8
TN20-208-33	Coprosma	Stems	96.9	5.7	4.4	46.0	35.6	59.5	9.5
TN20-208-34	Coprosma	Stems	96.8	5.5	4.4	44.6	34.0	59.3	9.5
TN20-208-35	Coprosma	Stems	96.7	5.1	3.1	47.7	36.8	58.9	9.4
TN20-208-36	Coprosma	Stems	96.8	6.2	4.4	39.6	30.5	62.2	10.0
TN20-208-37	Hoheria	Stems	96.5	9.5	7.5	50.1	40.5	55.2	8.8
TN20-208-38	Hoheria	Leaves	97.1	12.4	11.9	35.0	18.4	66.1	10.6
TN20-208-39	Hoheria	Leaves	97.0	12.1	12.5	33.0	18.8	64.8	10.4
TN20-208-40	Hoheria	Leaves	97.3	11.3	11.9	33.3	18.9	65.5	10.5
TN20-208-41	Hoheria	Leaves	97.7	11.1	8.8	37.9	20.4	65.6	10.5
TN20-208-42	Hoheria	Stems	95.5	10.8	8.8	47.7	38.1	54.4	8.7
TN20-208-43	Hoheria	Leaves	97.1	12.9	12.5	29.2	18.0	64.9	10.4
TN20-208-44	Hoheria	Stems	96.3	9.1	6.9	50.0	40.1	55.0	8.8
TN20-208-45	Hoheria	Stems	95.7	9.3	8.1	51.0	39.8	56.1	9.0
TN20-208-46	Hoheria	Stems	96.9	8.0	5.0	53.8	42.8	55.1	8.8
TN20-208-47	Pittosporum	Leaves	97.0	8.1	7.5	29.7	19.8	67.4	10.8
TN20-208-48	Pittosporum	Leaves	96.6	8.0	6.9	29.0	19.2	67.8	10.9
TN20-208-49	Pittosporum	Leaves	96.9	7.8	6.9	28.1	19.0	68.3	10.9
TN20-208-50	Pittosporum	Leaves	97.3	8.2	6.9	30.5	19.8	68.3	10.9
TN20-208-51	Pittosporum	Leaves	96.9	8.9	3.8	47.2	38.1	57.5	9.2
TN20-208-52	Pittosporum	Stems	96.7	9.0	5.0	44.8	36.0	57.1	9.1
TN20-208-53	Pittosporum	Stems	96.3	8.2	4.4	47.4	39.4	57.3	9.2
TN20-208-54	Pittosporum	Stems	97.3	8.3	4.4	50.6	40.2	57.9	9.3
TN20-208-55	Pittosporum	Stems	96.9	8.9	4.4	44.4	36.3	57.4	9.2
TN20-208-56	Pittosporum	Stems	97.3	8.1	5.6	31.0	17.8	69.2	11.1
TN20-898-01	Coprosma	Leaves	98.0	7.2	7.4	36.1	22.1	73.1	11.7
TN20-898-04	Salix	Leaves	97.7	5.1	16.6	35.2	26.4	67.8	10.9
TN20-898-05	Pittosporum	Leaves	97.6	6.5	5.9	37.2	20.8	71.6	11.5
TN20-898-06	Hoheria	Leaves	97.5	11.6	12.4	38.1	18.8	69.3	11.1
TN20-898-07	Griselinia	Leaves	98.0	7.7	5.4	30.2	22.1	72.4	11.6
TN20-898-09	Griselinia	Leaves	98.6	7.3	6.6	32.8	22.4	73.3	11.7
TN20-898-11	Salix	Leaves	98.4	4.9	14.8	34.3	23.9	69.3	11.1
TN20-898-12	Coprosma	Leaves	97.8	6.9	8.0	33.2	22.4	72.9	11.7
TN20-898-13	Pittosporum	Leaves	97.1	6.3	5.6	31.2	18.5	73.6	11.8
TN20-898-16	Hoheria	Leaves	96.6	11.7	13.0	33.7	21.3	67.8	10.9

Appendix 1: Wet chemistry results for native forage samples (continued).

NutLab ID	Species	Tissue	DM %	Ash %	CP %	NDF %	ADF %	In vitro DOMD %	ME
TN20-898-18	Salix	Leaves	97.6	4.8	13.7	33.0	22.6	69.3	11.1
TN20-898-20	Hoheria	Leaves	97.7	10.6	14.8	36.3	17.8	71.0	11.4
TN20-898-21	Pittosporum	Leaves	96.5	6.3	5.9	38.5	20.8	71.9	11.5
TN20-898-23	Griselinia	Leaves	97.8	6.9	6.4	30.6	21.1	73.8	11.8
TN20-898-24	Coprosma	Leaves	98.0	6.7	8.0	36.8	22.5	73.6	11.8
TN20-898-25	Coprosma	Leaves	97.7	6.6	7.8	40.5	20.8	74.2	11.9
TN20-898-26	Salix	Leaves	98.1	4.5	14.1	39.6	23.5	69.6	11.1
TN20-898-29	Pittosporum	Leaves	97.2	6.1	6.6	34.4	20.2	73.0	11.7
TN20-898-30	Hoheria	Leaves	97.6	11.2	14.2	38.1	20.6	69.3	11.1
TN20-898-31	Griselinia	Leaves	97.4	6.8	6.0	32.1	21.7	72.6	11.6
TN20-898-33	Coprosma	Stems	97.7	6.5	4.8	48.9	38.6	63.9	10.2
TN20-898-37	Salix	Stems	98.3	4.5	8.3	45.5	35.9	63.2	10.1
TN20-898-38	Pittosporum	Stems	97.9	6.7	4.4	50.1	39.3	59.9	9.6
TN20-898-39	Hoheria	Stems	97.9	9.2	8.1	50.7	37.9	59.1	9.5
TN20-898-40	Griselinia	Stems	98.7	6.3	4.9	50.7	41.2	63.5	10.2
TN20-898-42	Griselinia	Stems	99.6	5.7	3.2	47.8	37.3	63.2	10.1
TN20-898-43	Salix	Stems	98.9	3.4	7.7	47.5	37.1	64.1	10.3
TN20-898-44	Coprosma	Stems	97.9	6.4	4.9	45.4	36.7	61.6	9.9
TN20-898-45	Pittosporum	Stems	97.7	6.6	3.6	51.6	40.6	57.4	9.2
TN20-898-48	Hoheria	Stems	97.4	9.1	7.6	52.6	41.3	60.8	9.7
TN20-898-50	Salix	Stems	96.8	3.2	5.6	47.9	35.6	63.5	10.2
TN20-898-52	Hoheria	Stems	96.8	8.7	7.8	51.0	39.5	57.4	9.2
TN20-898-53	Pittosporum	Stems	97.0	6.8	3.8	51.1	40.4	58.7	9.4
TN20-898-55	Griselinia	Stems	97.5	4.9	3.9	46.7	37.2	59.8	9.6
TN20-898-56	Coprosma	Stems	97.4	6.1	4.4	43.0	33.7	62.6	10.0
TN20-898-57	Coprosma	Stems	97.0	6.0	4.3	44.5	34.3	60.3	9.6
TN20-898-58	Salix	Stems	96.9	3.3	5.9	50.0	41.3	58.9	9.4
TN20-898-61	Pittosporum	Stems	96.5	5.9	4.5	49.7	39.4	57.5	9.2
TN20-898-62	Hoheria	Stems	96.3	8.2	8.6	53.4	42.3	56.0	9.0
TN20-898-63	Griselinia	Stems	97.6	5.1	4.3	45.9	36.3	59.6	9.5
TN21-852-01	Coprosma	Stems	97.8	7.1	6.1	37.5	31.6	64.7	10.3
TN21-852-02	Coprosma	Leaves	98.1	7.1	9.3	34.9	23.2	74.3	11.9
TN21-852-03	Coprosma	Stems	97.8	7.0	5.9	37.6	28.4	64.8	10.4
TN21-852-04	Coprosma	Leaves	97.8	6.9	9.4	32.2	24.5	75.0	12.0
TN21-852-05	Coprosma	Stems	97.6	6.3	6.0	39.6	29.3	65.7	10.5
TN21-852-06	Coprosma	Leaves	98.6	6.8	9.4	32.6	21.9	75.9	12.1
TN21-852-07	Coprosma	Stems	98.0	6.1	6.2	41.6	30.6	65.0	10.4
TN21-852-08	Coprosma	Leaves	97.5	6.6	9.6	34.2	26.5	73.5	11.8
TN21-852-09	Griselinia	Stems	98.8	6.4	3.3	51.7	41.5	60.8	9.7
TN21-852-10	Griselinia	Leaves	98.6	7.2	7.0	30.6	19.8	74.9	12.0
TN21-852-11	Griselinia	Stems	98.1	6.0	3.1	49.7	37.9	58.0	9.3

Appendix 1: Wet chemistry results for native forage samples (continued).

NutLab ID	Species	Tissue	DM %	Ash %	CP %	NDF %	ADF %	In vitro DOMD %	ME
TN21-852-12	Griselinia	Leaves	98.4	6.5	6.8	27.9	20.8	74.2	11.9
TN21-852-13	Griselinia	Stems	98.5	5.7	3.8	49.5	39.8	60.8	9.7
TN21-852-14	Griselinia	Leaves	97.8	6.7	7.4	29.1	19.8	74.7	11.9
TN21-852-15	Griselinia	Stems	98.0	5.4	4.1	49.5	38.0	60.7	9.7
TN21-852-16	Griselinia	Leaves	97.0	6.5	7.4	32.4	21.0	74.3	11.9
TN21-852-17	Hoheria	Stems	97.1	10.2	8.8	47.1	38.3	53.1	8.5
TN21-852-18	Hoheria	Leaves	97.8	12.4	12.5	39.8	21.8	64.7	10.4
TN21-852-19	Hoheria	Stems	97.0	10.0	8.9	52.0	41.7	53.0	8.5
TN21-852-20	Hoheria	Leaves	97.6	13.2	13.8	35.7	19.9	63.1	10.1
TN21-852-21	Hoheria	Stems	96.8	9.6	8.1	50.8	40.1	52.5	8.4
TN21-852-22	Hoheria	Leaves	98.3	12.8	13.0	34.1	19.2	65.2	10.4
TN21-852-23	Hoheria	Stems	98.2	8.7	8.8	50.7	40.8	54.8	8.8
TN21-852-24	Hoheria	Leaves	98.5	12.8	13.5	33.4	19.1	65.7	10.5
TN21-852-25	Pittosporum	Stems	97.8	7.3	4.6	51.0	39.5	58.0	9.3
TN21-852-26	Pittosporum	Leaves	96.2	7.2	7.3	37.1	23.3	67.8	10.9
TN21-852-27	Pittosporum	Stems	97.3	7.4	4.2	47.8	36.1	58.9	9.4
TN21-852-28	Pittosporum	Leaves	96.7	6.8	6.6	29.3	20.9	71.0	11.4
TN21-852-29	Pittosporum	Stems	98.3	6.7	4.6	45.9	35.9	60.1	9.6
TN21-852-30	Pittosporum	Leaves	97.3	7.8	8.0	46.3	29.6	69.3	11.1
TN21-852-31	Pittosporum	Stems	97.1	7.3	4.0	51.6	38.9	58.9	9.4
TN21-852-32	Pittosporum	Leaves	95.3	6.5	7.4	34.0	21.7	70.4	11.3
TN21-852-33	Pseudopanax	Stems	97.4	10.0	3.0	33.9	26.3	64.9	10.4
TN21-852-34	Pseudopanax	Leaves	98.4	7.4	4.8	20.5	15.4	76.9	12.3
TN21-852-36	Pseudopanax	Leaves	97.8	7.4	5.6	20.9	16.4	75.2	12.0
TN21-852-37	Pseudopanax	Stems	97.1	8.2	4.4	35.6	27.2	65.0	10.4
TN21-852-38	Pseudopanax	Leaves	98.7	6.8	5.9	21.7	16.6	76.0	12.2
TN21-852-39	Pseudopanax	Stems	96.8	7.5	3.0	35.5	28.0	64.3	10.3
TN21-852-40	Pseudopanax	Leaves	98.7	6.4	5.8	22.4	17.6	75.4	12.1
TN21-852-41	Coprosma	Leaves	96.9	8.7	8.1	30.4	24.5	70.3	11.2
TN21-852-42	Coprosma	Stems	96.6	7.9	5.2	49.0	41.1	56.6	9.0
TN21-852-43	Coprosma	Leaves	96.9	7.7	9.3	37.6	28.7	69.9	11.2
TN21-852-44	Coprosma	Stems	96.2	6.7	5.9	51.4	43.0	55.9	8.9
TN21-852-45	Coprosma	Leaves	97.3	8.5	7.8	37.4	27.3	68.9	11.0
TN21-852-46	Coprosma	Stems	96.0	7.9	6.3	51.3	43.5	55.7	8.9
TN21-852-47	Coprosma	Leaves	96.9	10.5	9.3	35.4	23.2	69.2	11.1
TN21-852-48	Coprosma	Stems	96.4	6.6	6.9	51.8	42.6	55.3	8.8
TN21-852-49	Coprosma	Leaves	96.9	8.8	7.9	33.1	22.9	70.2	11.2
TN21-852-50	Coprosma	Stems	95.8	9.1	5.8	43.7	35.8	58.2	9.3
TN21-852-51	Mahoe	Leaves	96.8	10.4	12.4	44.0	27.6	61.2	9.8
TN21-852-52	Mahoe	Stems	96.9	6.4	8.5	67.0	57.5	43.5	7.0
TN21-852-53	Mahoe	Leaves	96.5	14.4	10.5	46.5	28.7	58.3	9.3

Appendix 1: Wet chemistry results for native forage samples (continued).

NutLab ID	Species	Tissue	DM %	Ash %	CP %	NDF %	ADF %	In vitro DOMD %	ME
TN21-852-54	Mahoe	Stems	94.8	10.3	6.4	67.1	58.3	41.5	6.6
TN21-852-55	Mahoe	Leaves	97.0	15.4	10.6	45.5	30.7	56.5	9.0
TN21-852-56	Mahoe	Stems	96.3	11.0	6.2	65.4	56.4	42.5	6.8
TN21-852-57	Mahoe	Leaves	97.1	11.4	9.5	45.3	30.4	58.2	9.3
TN21-852-58	Mahoe	Stems	96.3	8.0	4.6	68.9	60.4	41.3	6.6
TN21-852-59	Mahoe	Leaves	96.8	12.1	8.0	47.1	33.1	57.0	9.1
TN21-852-60	Mahoe	Stems	95.2	8.6	4.8	63.3	52.0	44.7	7.2
TN21-852-61	Pittosporum	Leaves	97.1	9.9	8.8	37.2	22.8	67.8	10.9
TN21-852-62	Pittosporum	Stems	97.1	7.5	4.0	60.5	51.6	48.8	7.8
TN21-852-63	Pittosporum	Leaves	97.7	9.7	7.9	34.8	23.7	66.7	10.7
TN21-852-64	Pittosporum	Stems	97.7	10.2	4.6	55.6	40.8	53.5	8.6
TN21-852-65	Pittosporum	Leaves	96.7	8.8	6.8	36.2	22.9	67.8	10.9
TN21-852-66	Pittosporum	Stems	97.2	7.8	3.9	56.8	47.3	51.8	8.3
TN21-852-67	Pittosporum	Leaves	96.5	9.1	8.2	28.6	21.1	67.9	10.9
TN21-852-68	Pittosporum	Stems	97.4	10.7	6.0	49.3	37.6	54.6	8.7
TN21-852-69	Pittosporum	Leaves	97.5	6.8	9.0	40.6	23.5	68.7	11.0
TN21-852-70	Pittosporum	Stems	97.0	7.9	5.6	52.2	41.5	53.8	8.6