Factors Affecting the Quality Characteristics of Black Wattle (*Acacia mearnsii* de Wild.) Bark

by

Preesha Avadianund Bridglall

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School of Agricultural, Environmental and Earth Sciences

College of Agriculture, Engineering and Science

University of Kwazulu-Natal

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Thesis Summary

Black wattle (*Acacia mearnsii* De Wild) stands as a pivotal commercial tree species, spanning 82,000 hectares of land in South Africa. The revenue stream, sourced the from timber (85 %) and bark (1 5 %) sales, is estimated at $200 million annually. Bark harvested from these trees undergoes processing at three factories in South Africa. The resultant products, namely "Mimosa Extract Powder" and "Mimosa Solid," serve vital roles in the global leather tanning sector, facilitating an annual export of roughly 45,000 tons of bark extract, valued at around $60 million. Typically, these trees reach their optimal rotation age at 10 years, ensuring peak wood and tannin yields. Though historical research primarily concentrated on timber and tannin yields, scant attention was paid to other factors affecting bark and extract quality, notably colour. Various environmental, genetic, and physical factors, alongside logistical and extraction processes, influence bark tannin content and colour. Post-harvest procedures involve quality grading and transportation to factories, where wattle bark undergoes industrial autoclave extraction to yield an extract liquor. Subsequently, this liquor is concentrated and either spray-dried to produce extract powder or evaporated into a solid, toffee-like product. This review aims to delve into the mechanisms and factors affecting tannin composition and colour degradation in both the raw material (wattle bark) and the final extract powder. The focus lies on establishing a foundation for developing mitigation strategies to bolster the quality of wattle bark and its extract. Such efforts are imperative for maintaining wattle extracts' market presence in the tanning industry and ensuring the sustainability of South Africa's wattle industry.

Tannins are the major extractable component of black wattle (*Acacia Mearnsii* de Wild) bark. In a wattle factory, extracted tannin levels are one of several quality parameters (Extraction efficiency and insoluble content are some of the other parameters) that are monitored, typically using a modified Soxhlet/ reflux extraction method. However, this method is slow and laborious, and the extracted product is unsuitable for tannin color analysis, which is another important product quality parameter. A new method was then developed, aiming to measure both the conventional bark parameters as well as the color of the extract solution, and increase sample throughput. The effect of oven-drying versus freeze-drying was

compared as a method of moisture removal for tannin color preservation prior to extraction. Sample filtration methods, extraction times, and chemical additives were also tested, compared, and optimised as necessary. Suitably processed bark samples were subjected to four extraction methods, i.e., Soxhlet, cold water, pressure cooker, and autoclave extractions. Extract properties were analysed using the standard Society for Leather Technologists and Chemists (SLTC) methods. Freeze-drying was shown to arrest color development in the bark. Deionised water was the best extractant tested, although the addition of ethylenediaminetetraacetic acid (EDTA) enhanced the color of the extracts. The autoclave extraction method, involving two extractions and centrifugation, was found to be the most practical and effective for wattle bark. It minimizes color change, reduces sample analysis variability, and allows for high sample throughput. This method is valuable for future research on wattle bark properties and for routine quality analysis in wattle extract factories.

Since the early 1900s, the Society of Leather Technologists and Chemists (SLTC) has used the hide powder method to evaluate tannin content and quality traits in wattle bark and Mimosa products. This approach has become the global standard in both the wattle bark and leather industries. Alternative methods, such as the Stiasny method, are predominantly employed for estimating adhesive tannin content, and ultraviolet (UV) spectroscopy is applied in both adhesive and tanning processes. However, Near-Infrared Reflectance Spectroscopy (NIRS) has emerged as a highly efficient, cost-effective, and non-destructive analytical tool. NIRS is widely used across various industries, including food and agriculture, and has proven its effectiveness for tannin analysis. This study explored the application of NIRS using different sample preparations, such as milled freeze-dried bark and bark extract solutions. By integrating NIRS scans with traditional SLTC methods and elemental analysis, models were developed to predict key properties of wattle bark and its extracts. UV spectroscopy was also tested for tannin determination but showed limited accuracy compared to NIRS. Notably, the NIRS analysis of milled freeze-dried bark demonstrated strong predictive performance for all critical quality parameters. These findings underscore the potential of NIRS as a viable alternative to traditional wattle bark analysis techniques. Its accuracy, speed, and non-

destructive nature make it a promising replacement for existing methodologies, offering enhanced efficiency for tannin content and quality analysis in the industry.

Black wattle (*Acacia mearnsii De Wild*) is a key tree crop in South Africa, valued for its bark and timber, both significant contributors to export revenue. Wattle bark harvesting begins with the rainy season in September and extends to May. During this time, harvested bark is transported to three processing facilities. The journey, which includes stripping and transit lasting several days, exposes the bark to varying environmental conditions, such as temperature, rainfall, humidity, and light, all of which can influence the quality of the bark. The decrease in extractives, darkening of the bark, loss of tannins are all examples of bark quality degradation. To replicate post-harvest conditions, experiments were conducted using fresh bark samples collected bi-monthly from September 2020 to July 2021. These samples were subjected to varying temperature, light, and moisture conditions to simulate real-life scenarios and quantify the extent of bark quality degradation. Quality parameters, including total extractives, tannin content, and Lovibond color (red), were analysed. Advanced statistical techniques, such as principal component analysis (PCA) and redundancy analysis (RDA), were used to identify patterns and relationships among variables. The findings revealed that seasonal changes and site-specific conditions significantly influenced bark quality, particularly affecting Lovibond colour, a key quality indicator. This study underscores the impact of pre-extraction environmental conditions on the quality of bark extractives. Developing strategies to mitigate these effects is essential to minimize variability and ensure consistent production of high-quality products. The study also highlights the need for more in-depth and future work.

In South Africa black wattle (*Acacia mearnsii* De Wild) and green wattle (*Acacia decurrens* var. *dealbata* (Link) F. Muell) are the only two species of wattle that are cultivated commercially. Green wattle has superior quality as a timber crop whereas black wattle produces superior tannin extracts for leather tanning applications. The cultivation of green wattle was actively discouraged by the wattle industry, until 2013 the widespread death of black wattle trees as a result of infection by wattle rust (*Uromycladium acaciae* (Cooke) P. Syd. & Syd.) inadvertently stimulated the increased production of green wattle in mixed plantations. This study aimed to determine the maximum quantity of green wattle bark that can be mixed with black

wattle bark without compromising the quality of the wattle extract products. Using mixtures of green and black wattle bark ranging from 0 % to 100% of each, the quality of extracts from bark mixtures were tested for the standard SLTC quality parameters. The addition of up to 40 % green wattle bark to black wattle bark had negligible effects on the quality of bark extraction liquor. Two Near Infrared Reflectance Spectroscopy (NIRS) models were developed for wattle bark mixtures. A Qualitative (IDENT) model was developed to detect the presence or absence of green wattle bark in consignments of largely black wattle bark. A Quantitative model was developed to quantify the levels of green wattle in mixtures of green and black wattle bark. Both models were successful, allowing for the detection of green wattle bark, and the accurate prediction of the level of green wattle bark in green and black wattle bark mixtures. Using NIRS to monitor green wattle bark levels, wattle extract factories will be able to use up to 40 % green wattle bark in mixed bark consignments without compromising the quality of the tannin extracts. This will benefit wattle farmers, who will be able to increase their timber revenue without reducing their bark revenue by increasing the percentage of green wattle to black wattle up to 40 % of each plantation.

Black wattle (*Acacia mearnsii* de Wild.) is an important and high-value South African forestry species for bark and timber production. One of the objectives of the wattle breeding programme at the Institute for Commercial Forestry Research (ICFR) is to improve bark quality. Evaluating bark quality in breeding trials requires sampling of large numbers of trees, without causing any damage to the valuable wattle trees. Therefore, traditional wet-chemistry lab methods are impractical because of they are slow, expensive and require destructive sampling. Thus, the ICFR breeding programme would benefit from a rapid method to assess bark quality non-destructively. This study employed a novel Near Infrared Spectroscopy (NIRS) rapid and semi-destructive screening protocol to assess the quality of bark in field trials of black wattle. The protocol was used to compare wattle bark quality parameters of trees in block trials, established using various seed sources and clonal material. The predictive models developed showed a high degree of accuracy across the various parental trees and clonal varieties. The study showed that bark quality parameters across six different seed sources at a block trial in Luneburg showed very little variation, whereas bark from the same seed sources in another block trial in Iswepe

(Creydt) showed differences to each other and were different to the same sources planted in Luneburg. In the clonal trials, Tannin % and Lovibond colour showed more variation at Schwarzwald, (CLT2) than at Harden Heights (CLT3), with SP1-35 being one of the best performing clones at both Schwarzwald and Harden Heights. Site-by-genotype interaction analysis revealed that certain clones performed better in specific soil conditions. These findings can assist in guiding seed source/clone selection and site matching strategies, ensuring that the genetic potential of black wattle germplasm is maximised under the most suitable growing conditions. Overall, the development of the NIRS model can contribute to a more targeted and efficient breeding process and to the sustainable development of black wattle plantations.

The project was initiated to identify key factors influencing black wattle bark quality, with the aim of providing mitigation strategies. As a result, robust analytical methods for bark quality assessment were developed, offering significant value to the black wattle bark extract industry. The research also highlighted critical areas for future investigation, including long-term monitoring of site-specific physical and environmental variables. A notable outcome was the demonstrated robustness and versatility of near-infrared spectroscopy (NIRS), which showed strong potential for various applications such as breeding selection, species differentiation, routine quality monitoring, and broader research use. Continued research would be recommended, as the current findings lay a strong foundation for future studies that could produce favourable benefits for the industry.

Signed: [redacted] Date: 12/07/2025

Preesha Avadianund Bridglall

Signed: [redacted] Date: 11/07/2025

Dr Richard J. Burgdorf

Signed: [redacted] Date: 14 / 07 / 2025

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Dedication

I would like to dedicate this work to my precious daughters Saheli and Shristi Bridglall

Let the beauty of what you love, be what you do……. Rumi

Declaration 1: Plagiarism

I, Preesha Avadianund Bridglall declare that:

1) The work in this thesis, except where otherwise indicated or acknowledged, is my original work.

2) This thesis has not been submitted in full or in part for any degree or examination to any other university.

3) This thesis does not contain other persons' data, pictures, graphs, or other information unless specifically acknowledged as being sourced from other persons.

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Date: 12/07/2025

Declaration 2: Publication

The following work has been published in a peer-reviewed, ISI-accredited scientific journal:

Chapter 2: A novel laboratory method for the extraction of black wattle (*Acacia mearnsii* de Wild.) bark constituents. 2025. P. Avadianund Bridglall, M.D., Laing, N. Naidoo, R.J., Burgdorf. Journal of American Leather Chemists Association.120(10), pp 26 – 41.

Chapter 4: Ageing, climatic, physical, and site effects on black wattle (*Acacia mearnsii* de Wild.) bark. 2025. P. Avadianund Bridglall, M.D., Laing, C. Morris, R.J., Burgdorf. View online at Wiley Online Library (wileyonlinelibrary.com); DOI:10.1002/bbb.2795; *Biofuels, Bioproducts and Biorefining.* Pp 1- 17.

Chapter 1: A review of the factors affecting black wattle (*Acacia mearnsii* De Wild.) bark quality in South Africa. 2025. P. Avadianund Bridglall, M.D., Laing, A. Morris, R.J., Burgdorf. Southern Forests: A Journal of Forest Science. (Accepted April 2025). In Press.

The following work has been submitted to peer-reviewed, ISI-accredited scientific journals and is currently under review:

Chapter 3: Near-Infrared Spectroscopic (NIRS) analysis of black wattle (*Acacia mearnsii* de Wild.) bark quality parameters. 2025**.** P. Avadianund Bridglall, M.D., Laing, R.J., Burgdorf. Journal of the Science of Food and Agriculture. Submitted May 2025.

For all published and submitted work, my contributions included formulating the hypotheses, identifying the research problems, determining the experimental approach, conducting the experiments, collecting the data, performing the statistical analyses, and writing the text.

The co-authors contributed by assisting with experimental design, statistical analyses, and reviewing the document.

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List of abbreviations and acronyms

° C	Degrees Celsius
$	Dollar
%	Percentage
Al	Aluminium
ANOVA	Analysis of variance
B	Boron
BW	Black wattle
C	Carbon
$C_2H_2O_4$	Oxalic acid
$C_2H_7NO_2$	Ammonium acetate
$C_6H_8O_6$	Ascorbic Acid
Ca	Calcium
CAGR	Compound annual growth rate
CH_3COOH	Acetic acid
cm	Centimetres
Cu	Copper
CV	Coefficient of variation
Da	Dalton
DAW	Double autoclave water method
DBH	Diameter at breast height
DRIS	Diagnosis and recognition integrated system
EDTA	Ethylenediaminetetraacetic acid
EMS	Electrospray mass spectrometry
ER	Extraction ratio
ESI-MS	Electrospray ionization mass spectroscopy
EVP	Evapotranspiration
FSA	Forestry South Africa
FSC	Forest Stewardship Council
g	Gram/s
GC	Gas chromatography
GDP	Gross domestic product
GW	Green wattle

H_2O	Water
ha	Hectare/s
HMA	Halogen moisture analyser
ICFR	Institute for Commercial Forestry Research
IQ	Index of quality
IQR	Interquartile range (IQR)
Lins CCC	Lins Concordance Coefficient
LIRI	Leather Industries Research Institute
LRU	Lovibond red units
LYU	Lovibond yellow units
K	Potassium
KCl	Potassium chloride
m	Metre
M	Molar
mf	Moisture factor
Mg	Magnesium
mL	Millilitre
Mn	Manganese
mm	Millimetre
MSC	Multiplicative Scatter Correction
mTorr	Millitorr
m/v	Mass to volume ratio
n	Number of samples
N	Nitrogen
Na	Sodium
NaOH	Sodium hydroxide
NIR	Near infrared reflectance
NIRS	Near infrared reflectance spectroscopy
NITS	Near infrared transmission spectroscopy
nm	Nanometre
NMR	Nuclear magnetic resonance
No.	Number
NTU	Nephelometric turbidity unit

OEC	Observatory for Economic Complexity
P	Phosphorus
PACs	Proanthocyanidins
PCA	Principle component analysis
PERMANOVA	Permutation multivariate analysis of variance
PSLR	Partially Squared Linear Regression
R^2	Coefficient of determination
R & D	Research and development
RDA	Redundancy analysis
RHP	Relative humidity
RMSECV	Root-mean standard error for the cross-validation
RMSEP	Root means square error of prediction
RPD	Relative percentage difference
RPIQ	Ratio of Performance to Interquartile Distance
rpm	Revolutions per minute
S	Sulphur
SD	Standard deviation
SLTC	Society for leather technologists and chemists
spp.	Species (plural)
SNV	Standard Normal Variate
SRAD	Solar radiation
T/NT	Tannin/Non-Tannin Ratio
USD	United States Dollar
UV	Ultraviolet
V	Volume
WRI	Wattle Research Institute
Zn	Zinc

General introduction

Forestry offers a wide array of benefits, including economic, environmental, social, and cultural advantages (Freer-Smith and Carnus, 2008). Globally, forests cover over 30% of the total land area, amounting to 4.06 billion hectares, with plantation forests accounting for 3.8% of this area (FAO and UNEP, 2020). Plantation forestry primarily serves for wood production used in construction, paper, fiber, as well as fuel for cooking and energy (Sands, 2013). Additionally, non-wood uses include food, pharmaceuticals, tannins, dyes, cosmetics, chemicals like resins and oils, and ornamental plants.

Forestry holds significance both on a global and local scale, particularly in remote or rural areas where economic opportunities are limited (Raugh *et al.,* 2020). In South Africa, despite covering only 1% of the total land area, plantation forests span approximately 1.2 million hectares. These forests are distributed across five out of the nine provinces in the country. The breakdown of South Africa's land area reveals that grazing occupies 68.6%, arable land 13.9%, nature conservation areas 9.6%, and the remaining 6.9% is categorized as 'Other'. The map of South Africa depicts the primary activities related to plantation forestry across the nation (Figure 1).

Figure 1 A map of South Africa that illustrates the plantation forest areas (Source : https://www.forestrysouthafrica.co.za/info-graphics/homepage/introducing-commercial-forestry/).

The South African forestry sector contributes as much as 1.9% towards the Gross Domestic Product (GDP) of the country, with an average of R25 billion in annual exports. The sector provides 92,700 direct and indirect jobs (Upfold *et al.,* 2015). The various industries associated with forestry are illustrated in Figure 2.

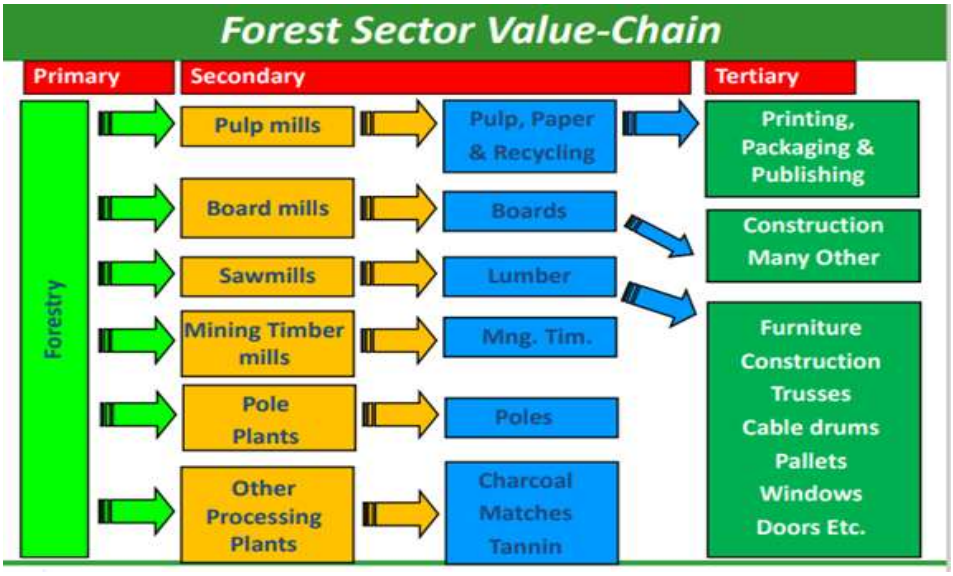


Figure 2 The forestry sector value chain in South Africa (Source: Oberholzer, 2021).

In South Africa, trees cultivated for commercial forestry purposes primarily belong to three main groups: exotic species originating from Australasia (*Eucalyptus/Corymbia* spp. and *Acacia* spp.) and North America (*Pinus* spp.). *Eucalyptus* species are valued as hardwood species, essential to the paper and pulp industry, while pine species (*Pinus* spp.) yields softwood predominantly used in timber production. Wattle (*Acacia* spp.) is a hardwood variety cultivated for both the wood pulp and wattle bark industries (SAPPI, Online (https://cdn-s3.sappi.com/s3fs-public/Part-2-Silviculture.pdf)). Figure 3 illustrates the scale of these species planted for forestry purposes.

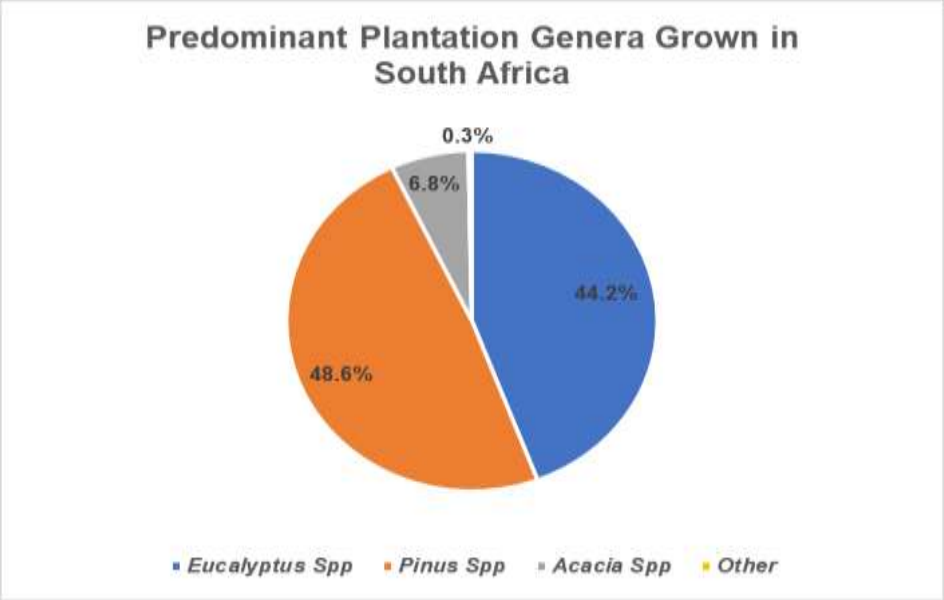


Figure 3 The composition of plantation species in South Africa (Source: Oberholzer, 2021).

Figure 3 illustrates that *Acacia* spp. constitutes a relatively small portion of South Africa's plantation forestry, at 6.8%. Black wattle, predominantly cultivated in KwaZulu-Natal (68,406 ha) and Mpumalanga (11,827 ha), also has a limited presence in Limpopo (20 ha), Eastern Cape (1,659 ha), and Western Cape (31 ha) (Oberholzer, 2021).

Wattle timber and bark extract products play a significant role in exports, generating an estimated $200 million (USD) in revenue. The timber is favoured by the paper and pulp industries, as well as being utilized for firewood, charcoal, and treated poles (Chan *et al.,* 2015). Green wattle (*Acacia decurrens* var. *dealbata* (Link) F. Muell.) is also cultivated for timber, although its bark is less suitable for the wattle bark industry due to its low tannin content and poor colour quality (Searle, 1997).

The wattle bark industry is centered around three factories in South Africa (two in KwaZulu-Natal and one in Mpumalanga). These factories process freshly harvested black wattle bark into extracts primarily used for leather tanning and adhesive production (such as Bondtite®) (Havemann, 1992). Ongoing exploration into the "green" or "bioeconomy" potential of wattle bark includes its use as an alternative to synthetic chemicals in mining, pharmaceutical production, water treatment, and enhancement of animal feed production (Ogawa and Yazaki, 2018; Das *et al.,* 2019).

Given its export potential, safeguarding and even expanding the market share of black wattle bark extracts is crucial. However, market share stability hinges on factors such as bark availability and consistent quality (Chan *et al.,* 2015).

Problem statement

Currently, there is a lack of information regarding the determinants of bark quality, underscoring the need for dynamic management approaches to assess any decline in bark quality, and to find ways to enhance the quality of bark extracts. Potential factors influencing bark quality include environmental aspects such as climate change, genetic considerations such as the introduction of new clonal material or seed sources, plantation management practices, the inadvertent cultivation of green wattle trees amongst black wattle plantations, and a range of bark processing techniques encompassing bark harvesting and transportation, which can contribute to bark degradation.

Despite their importance, relatively little research has been conducted on these factors and their correlation with the quality of bark extract products. Consequently, any factor causing a deterioration in bark quality usually causes a decline in the quality of bark extract, adversely impacting its market share for tanning products. While historical research on bark extracts has primarily focused on tannin yield, other factors significantly influence their market value, notably the colour of extracts.

Additionally, threats to the sustainability of the wattle bark industry include a reduction in the land area dedicated to black wattle cultivation, influenced by various factors such as the encroachment of invasive species and the negative impact of new pest and diseases. This decline in land area is illustrated in Figure 4.

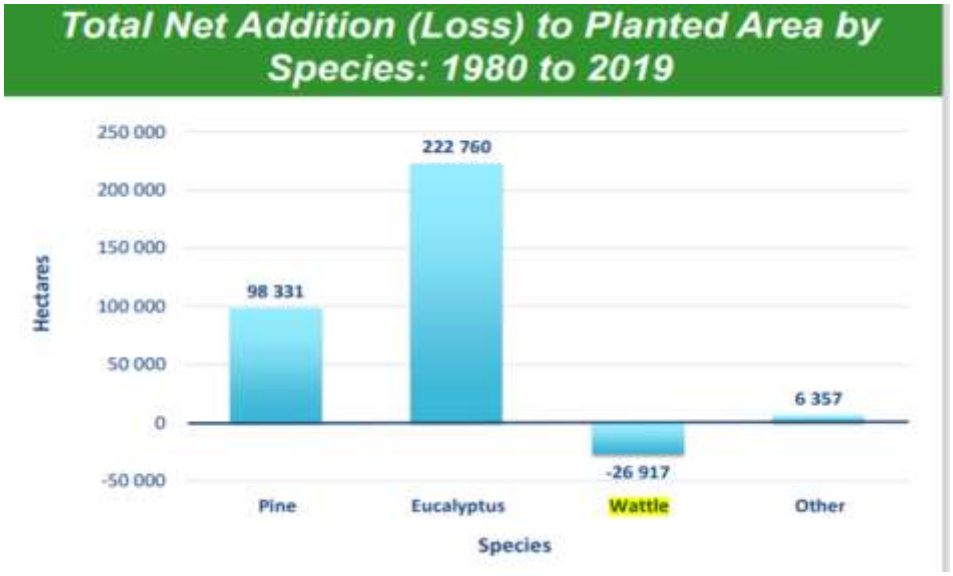


Figure 4 Changes in the planted area of the different species in South Africa (Source: Oberholzer, 2021).

Maintaining the highest quality standards for black wattle bark extract products is crucial for the industry. A decrease in product quality could diminish the value for growers, leading to a further reduction in planted area. Consequently, this could create a shortfall in meeting the demand for wattle bark and timber volumes.

Purpose of the study

Initiated in 2019, this research was part of a collaborative project between UCL and NTE (formerly **U**nion **C**o-operative **L**imited and **N**atal **T**anning **E**xtracts) and was funded by both companies and the government's Forestry Sector Innovation Fund (FSIF), managed by Forestry South Africa (FSA). The decision of the competing companies to collaborate in this research project stemmed from their joint concerns about the future supply and quality of wattle bark, potentially impacted by climate change and forestry trends. The stakeholders agreed on the following study objectives:

1. Enhance our understanding of the various factors influencing the quality of black wattle bark extracts.
2. Develop practical methods for the efficient monitoring and assessment of wattle bark arriving at extract factories, aiming for higher throughput without any loss of quality control.

3. Facilitate industry-relevant research and identify opportunities for improvement in silvicultural, breeding, harvesting, and processing practices, ultimately leading to superior quality tannin products and increased yields.

Aim and objectives of the study

The study aimed to explore the factors influencing the quality of black wattle bark. The objectives were twofold: firstly, to develop new rapid analytical methods for bark assessment, and secondly, to utilise these enhanced tools to examine the physical and environmental factors impacting bark tannin content and colour. The study was structured around several activities:

1. Conducting a comprehensive literature review encompassing wattle bark production, extract properties, and quality determination, serving as a valuable reference for the industry.
2. Developing a novel, efficient method for bark tannin extraction, capable of swiftly determining tannin content and colour quality, unlike the current labour-intensive Soxhlet-based reflux method.
3. Exploring Near-Infrared Reflectance Spectroscopy (NIRS) techniques for direct bark sample analysis, aiming to develop a technology for the rapid assessment of various quality parameters without the need for extraction, thus overcoming the limitations of existing methods.
4. Investigating environmental factors impacting black wattle bark colour quality through simulated aging experiments, assessing the effects of light, temperature, moisture, and their combinations on extracted bark tannin colour, alongside monitoring weather and soil properties over the harvesting season.
5. The study compares the quality differences between black and green wattle bark, utilising NIRS to distinguish between the two and to identify threshold levels at which green wattle bark compromises extract quality. The findings aim to support both growers and manufacturers, particularly in light of increasing demand for wattle timber, to which green wattle may contribute significantly.

6. Employing NIRS to assess the chemical/tannin quality of trees in breeding trials, aiming to understand site-by-gene interactions and to select superior genotypes for future planting.

These methodological advancements and research outcomes could assist the industry to implement and monitor mitigation strategies to prevent quality decline, and to uphold or enhance the high standard of bark extract products in South Africa.

Thesis format

The thesis format is in accordance one of the official University of Kwazulu-Natal formats, *i.e.,* a research paper format. All the chapters can be published as independent journal articles. Therefore, there will be some repetition of text or references, where it is relevant or necessary to the contents or a discrete chapter.

The thesis includes a Thesis Summary, which is the abstract of the six research chapters and contains a brief overview of the work done. The contents table, list of figures, list of tables, and list of abbreviations and acronyms are found after the Thesis Summary, and this is to illustrate the succeeding content. The General Introduction is a broad overview of the background and justification for doing this work and serves as the first chapter.

The six chapters encompass the problem's scope, the study's purpose, and the previously outlined aims and objectives. The first chapter is a review of the relevant literature focused on black wattle (*Acacia mearnsii*) bark quality, composition, the quality characteristics of the bark, as well as brief descriptions of the cultivation of wattle for wattle bark production, and the uses of tannins. This is followed by five experimental chapters, two of which are the development of methods required for the subsequent chapters. The Thesis Overview discusses the major findings of each experimental chapter, their impact, and future work that can be of value to the industry. Chapter 1,2, and 4 is presented as per the referencing style prescribed by the target peer-reviewed journal. Chapters 3,5, and 6 follow the University of KwaZulu Natal guidelines.

The experimental chapters contain an Introduction, Materials and Methods, Results, Discussion, and References sections, as per the format of a typical research paper.

Citations and references are in the author-date format using Harvard–style referencing. Data and statistical analysis that are chapter-specific, were performed in Microsoft Excel (Version 2207 Build 16.0.15427.20166), or in R (CRAN, version 4.2.0). Cinco (Version 5.12) was used for the principal component analysis (PCA) and redundancy analysis (RDA). Permutation multivariate analysis of variance (PERMANOVA) was performed using Primer (Version 6), and univariate analyses ANOVA done in Past 3 software (Version 3.20).

References

Chan, J.M., Day, P., Feely, J., Thompson, R., Little, K.M. and Norris, C.H. 2015. *Acacia mearnsii* industry overview: current status, key research, and development issues. *Southern Forests: A Journal of Forest Science*, *77*(1), 19-30.

Clarke, K.R. and Gorley, R.N. 2006. *PRIMER v6: User Manual/Tutorial (Plymouth Routines in Multivariate Ecological Research).* PRIMER-E, Plymouth.

Das, A.K., Islam, M.N., Faruk, M.O., Ashaduzzaman, M., Dungani, R., Roseman, E., Hartati, S. and Ruminated, A. 2019. Hardwood tannin: sources, utilizations, and prospects. In *Tannins-Structural Properties, Biological Properties and Current Knowledge*. London, United Kingdom. Pp 3-21.

Erbaugh, J.T., Pradhan, N., Adams, J., Oldekop, J.A., Agrawal, A., Brockington, D., Pritchard, R. and Chhetri, A. 2020. Global forest restoration and the importance of prioritizing local communities. *Nature Ecology & Evolution*, *4*(11), 1472-1476.

Freer-Smith, P. and Carnus, J.M. 2008. The sustainable management and protection of forests: analysis of the current position globally. *AMBIO: A Journal of the Human Environment*, *37*(4), 254-262.

FAO and UNEP. 2020. *The State of the World's Forests 2020*. Forests, biodiversity, and people. FAO, Rome. https://doi.org/10.4060/ca8642en

Garbutt, D.C.F. and Jones, R.A. 1979. *Comparative Tannin Analysis*. Wattle Research Institute, Pietermaritzburg. Pp 42-48.

Hammer, Ø., Harper, D.A.T., and Ryan, P.D. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica*, 4(1), 9.

Havemann, D.A. 1992. *The Manufacture and Uses of Wattle Bark Extract*. Ph.D. thesis, University of Stellenbosch. Pp 31-48.

https://www.forestrysouthafrica.co.za/info-graphics/homepage/introducing-commercial-forestry/. Accessed 1 September 2022.

Oberholzer, F, 2022. Personal communication with Mr. F. Oberholzer, Operations Manager, Forestry South Africa, Pietermaritzburg, August 2022.

Oberholzer, F. 2021. South African Forestry and Forest Products Industry 2019. https://www.forestrysouthafrica.co.za/info-graphics/homepage/introducing-commercial-forestry/. Accessed 1 September 2022.

Ogawa, S. and Yazaki, Y. 2018. Tannins from *Acacia mearnsii* De Wild. Bark: Tannin determination and biological activities. *Molecules*, *23*(4), 837.

Sands, R. (Ed.). 2013. *Forestry in a Global Context*. CABI, Wallingford, UK. Pp 1-37.

Searle, S.D. 1997. *Acacia mearnsii* De Wild. (Black wattle) in Australia. In: *Black Wattle and its Utilization.* Rural Industries Research and Development Corporation, Barton, ACT, Australia. Pp 1-10.

SAPPI. Undated, online. Tree Farming Guidelines for Private Growers. *Part 2*. *Silviculture.* SAPPI, Pietermaritzburg, South Africa. https://cdn-s3.sappi.com/s3fs-public/Part-2-Silviculture.pdf.

ter Braak, C.J.F. and Smilaxes, P. 2012. *Canoco reference manual and user's guide: software for ordination, version 5.0.* Microcomputer Power, Ithaca, NY.

Upfold, S.J., Dlamini, N. and Ndlela, N. 2015. Knowledge to support small-scale tree growers in South Africa. XIV WORLD FORESTRY CONGRESS, Durban, South Africa, September 2015. Pp. 7-11.

Chapter 1: A review of the factors affecting black wattle (*Acacia mearnsii* De Wild.) bark quality in South Africa

P. Avadianund Bridglall, M.D. Laing, A. Morris, and R.J. Burgdorf

1.1 Abstract

Black wattle (*Acacia mearnsii* De Wild) stands as a pivotal commercial tree species, spanning 82,000 hectares of land in South Africa. The revenue stream, sourced the from timber (85 %) and bark (15 %) sales, is estimated at $200 million annually. Bark harvested from these trees undergoes processing at three factories in South Africa. The resultant products, namely "Mimosa Extract Powder" and "Mimosa Solid," serve vital roles in the global leather tanning sector, facilitating an annual export of roughly 45,000 tons of bark extract, valued at around $60 million. Typically, these trees reach their optimal rotation age at 10 years, ensuring peak wood and tannin yields. Though historical research primarily concentrated on timber and tannin yields, scant attention was paid to other factors affecting bark and extract quality, notably colour. Various environmental, genetic, and physical factors, alongside logistical and extraction processes, influence bark tannin content and colour. Post-harvest procedures involve quality grading and transportation to factories, where wattle bark undergoes industrial autoclave extraction to yield an extract liquor. Subsequently, this liquor is concentrated and either spray-dried to produce extract powder or evaporated into a solid, toffee-like product. This review aims to delve into the mechanisms and factors affecting tannin composition and colour degradation in both the raw material (wattle bark) and the final extract powder. The focus lies on establishing a foundation for developing mitigation strategies to bolster the quality of wattle bark and its extract. Such efforts are imperative for maintaining wattle extracts' market presence in the tanning industry and ensuring the sustainability of South Africa's wattle industry.

Keywords: Black wattle, Mimosa Extract, South Africa, Quality

1.2 Introduction

Wattle trees, members of the Fabaceae family, belong to the genus *Acacia*, comprising over 1500 species worldwide (Ogawa and Yazaki, 2018; Dyer, 2014). South Africa documents more than 114 species (Magona et al., 2018).

Two species, black wattle (*Acacia mearnsii)* and green wattle (*Acacia decurrens var. dealbata (Link) F. Muell)* are cultivated commercially. In South Africa, black wattle is the only species with significant economic value for both its bark and timber (Dunlop and MacLennan, 2002).

Black wattle bark, rich in tannins, is used in leather tanning and adhesives due to its solubility and low viscosity (Gujrathi and Babu,2007). Other applications include firewood, charcoal, animal feed, pharmaceuticals, water treatment, dyestuffs, and mining (Chaunbi, 1997).

1.2.1 History of the wattle industry

In the 18th century, several wattle species were introduced from Australia to South Africa for sand dune stabilization in the Cape Province. Among the first species introduced were Coastal Wattle (*Acacia cyclops* A. Cunn. ex G. Don), Sydney Golden Wattle (*Acacia longifolia* (Andrews) Wild), and Golden Wreath Wattle (*Acacia saligna* (Labill.) H.L. Wendl.), also known as Port Jackson. In the 19th century, additional wattle species were introduced primarily for ornamental purposes. Later, black wattle (*Acacia mearnsii*), green wattle (*Acacia decurrens*), and Australian blackwood wattle (*Acacia melanoxylon* R. Br.) were brought in for timber production (Stephens, 1940).

A major development in wattle cultivation was the establishment of the tannin industry, focusing on the tannin compounds in the bark, which serve as a plant defence mechanism (Makkar and Becker 1998). Black wattle and South American Quebracho (*Schinopsis lorentzii* Engl.) became the primary global sources of natural tannins used in leather tanning (Hillis, 1997).

Black wattle's use in leather tanning dates back over 200 years, with the first reference in Australian literature circa 1824. Seeds were introduced to South Africa in 1864 by John Vanderplank, and by 1884, Lyle's Tannery in Pietermaritzburg conducted tanning tests with black wattle bark (Beard, 1957). The first tannin factory

opened in Pietermaritzburg in 1918, establishing the wattle bark industry (Sherry, 1971). Black wattle continues to be cultivated in KwaZulu-Natal and Mpumalanga (Chan *et al.,* 2015) as shown in Figure 1.1.

Figure 1.1 A typical black wattle (*A. mearnsii*) plantation at Harden Heights, in KwaZulu-Natal (GPS coordinates 29.2667° S, 30.6167° E) (Source: Bridglall).

Following World War I, wattle plantations in South Africa rapidly expanded due to its value as a crop and its ability to reduce soil erosion, a concern for government and forestry officials (Stubbings, 1977).

1.2.2 Black wattle plantations and wattle demand in South Africa

Wattle trees not only mitigate soil erosion but also enhance sustainable agriculture through nitrogen fixation in symbiosis with rhizobial bacteria, promoting rapid growth (Chaunbi, 1997). Planted in monocultures for consistent timber and bark, wattle plantations face increased disease risks, potentially leading to significant losses (Roux *et al.,* 1975). Additionally, unmanaged "jungle wattle," such as silver wattle (*Acacia dealbata*), poses ecological challenges by consuming water in dry areas and affecting rural livelihoods (Campbell *et al.,* 1999). Predominantly found in the Eastern Cape and KwaZulu-Natal, aggressive thinning of these dense thickets can restore ecosystems for viable management (Clarke, 2018).

Black wattle accounts for nearly 7 % of South Africa's timber planting area, with approximately 82,000 hectares dedicated to its cultivation (Tewari, 2001). Historical plantation rates for black wattle from 1980 to 2018 are shown in Figure 1.2, based on FSA data.

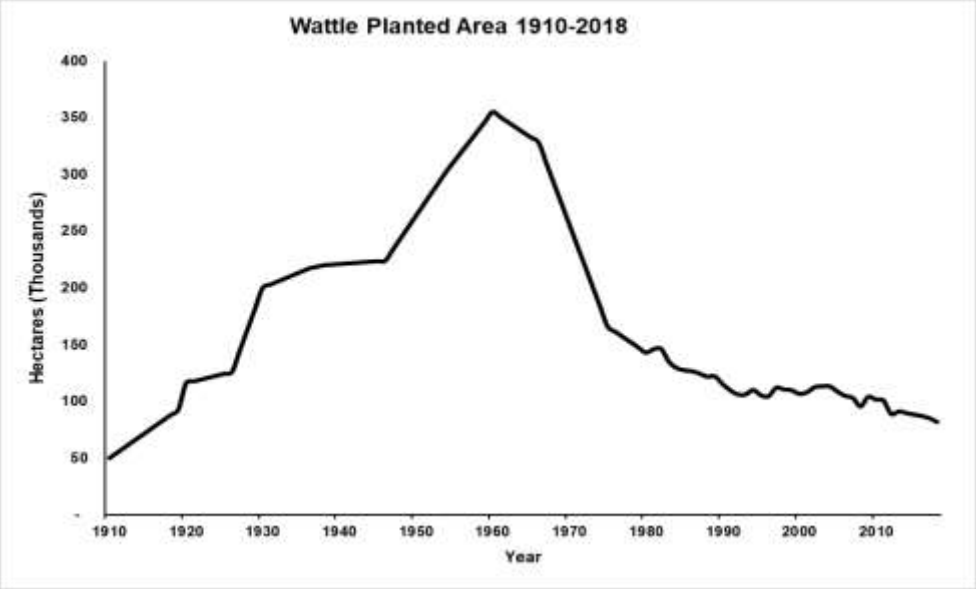


Figure 1.2 The wattle planted area in South Africa from 1918 to 2018 (Morris, 2022).

In the early to mid-1900s, the vegetable tanning and leather industries thrived, with black wattle bark and extract being dried and exported for tannin production (Craib, 1941). However, since the 1960s, black wattle planting has declined due to several factors. In 1951, an oversupply of wattle resulted from increased demand, leading to expanded afforestation. Bark prices reached their peak, but by 1958, when the first plantations were ready for felling, the supply of wattle exceeded demand. This imbalance prompted the introduction of a quota system, significantly reducing growers' income (Sherry, 1973).

The situation worsened with the global financial crisis of the 1960s, which reduced demand for leather products. This, in turn, led to lower tannin prices and decreased sales (Wade, 2015). Faced with these financial challenges, wattle plantations were gradually replaced by *Eucalyptus* and *Pinus* species, which offered higher timber yields and were more versatile for industries like construction and paper production (Searle, 1997). These changes in market demand and economic incentives led to the subsequent decline wattle timber tonnage since 2015 as illustrated in Figure 1.3.

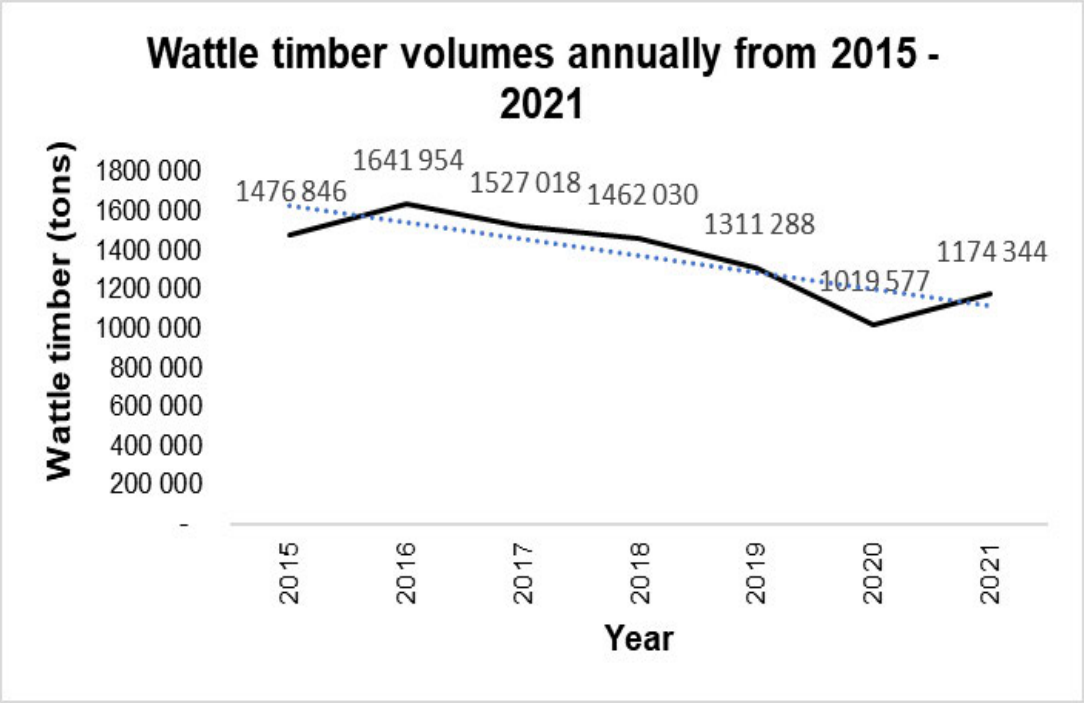


Figure 1.3 Annual production of wattle timber in South Africa between 2015 and 2021. (Source, FSA, 2022). The solid line represents the timber volumes, and the dotted line indicates a downward trend.

Figure 1.3 illustrates the collective wattle timber production, reported by FSA members who represent approximately 93 % of the industry. The planting cycle is driven by price dynamics and wood chip export demand, with bark often treated as a by-product of tree felling for wood chips (Morris, 2022). Black wattle's high wood density and pulp yield give it an economic edge in pulp production and storage, particularly for dissolving pulp and chemical cellulose. Kraft pulp in Japan largely uses black wattle woodchips from South Africa and Brazil (Chan *et al.,* 2015).

Despite a 5.11 % decline in the wattle extract market (OEC World), predictions for the European leather tanning industry show a promising Compound Annual Growth Rate (CAGR) of 5.7 % through 2030 (Chaudhary and Prasad, 2021). To maintain and expand South Africa’s market share, product quality remains an important factor.

In 2022, South Africa contributed 35 % to global wattle extract sales and held 46 % of the market share in 2019, positioning it as the largest exporter of wattle extracts (The Observatory for Economic Complexity (OEC World, 2024, https://oec.world/)). However, the industry has faced challenges such as increased competition from chromium tanning compounds (Chan *et al.,* 2015) and other vegetable tanning

agents like Quebracho and Chestnut (Pizzi, 2008). Market trends have also shifted, with decreasing demand for sole leather and growing importance placed on light-fast leather (Kenea, 2022).

The wattle industry is further threatened by the rising popularity of vegan and synthetic leather alternatives (Dunlop and MacLennan, 2002) and the growing demand for synthetic tannins derived from fossil fuels (Pinto *et al.,* 2013). To ensure the industry's growth and sustainability, the quality of bark and powder extracts must be prioritised, as only the highest quality products will be competitive in the evolving market.

1.3 Aim of the literature review

This review examines the historical significance and economic importance of black wattle bark in South Africa. It discusses both global and local market trends, the extraction process from silviculture to final extract, and the challenges encountered by growers and factories. The aim is to underscore the pivotal role of wattle bark in the forestry sector while shedding light on existing threats and potential opportunities. Additionally, the review offers recommendations for the future sustainability and growth of the wattle industry.

1.4 Review methodology

The literature cited in this review has been collected from various sources. Most of the literature was sourced from the Institute for Commercial Forestry Research (ICFR). Personal communications between NTE (Pietermaritzburg) and UCL (Dalton) also played a significant role in the context of the literature collected and reviewed. Google Scholar was also used to locate more recent literature as the literature sourced from the ICFR was over 20 years old. Online web articles such as SA Forestry online also provided information for this review.

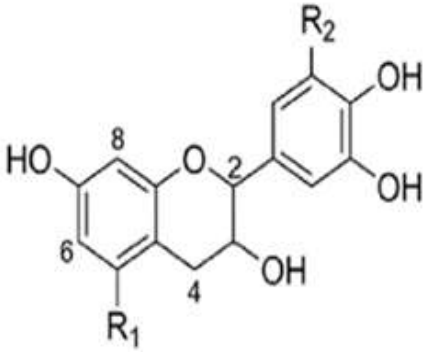
1.5 Natural Tannins

Tannins, astringent polyphenols found in many plants, are known for their protein-binding properties, which contribute to their bitter taste (Haslam, 1989). They play a significant role in the flavour and stability of beverages like tea and wine, with wine tannins providing anti-oxidative benefits that enhance colour and structural stability (Joslyn and Goldstein, 1964).

Tannins are generally classified into two groups: hydrolysable tannins (also known as pyrogallols including gallotannins & ellagitannins), and condensed tannins (known as catechols) (Khanbabaee and Van Ree, 2001). Hydrolysable tannins are derived from trees such as *Castanea*, *Terminalia*, *Phyllanthus*, and *Caesalpina* species and consist of gallic acid units linked to sugars by ester bonds (Pizzi, 2008). Condensed tannins, the focus of this review, are found in species like mimosa (*Acacia* sp.), Gambier (*Uncaria* sp.), quebracho (*Schinopsis* sp.), hemlock (*Tsuga* sp.), and pine (*Pinus* sp.) (Hills, 1997).

1.5.1 Condensed Tannins

Black wattle bark tannins are essential in the leather industry, binding proteins in animal hides to stabilize collagen fibres and prevent disintegration (Evelyn, 1957). They are also utilized in epoxy adhesives, where the aromatic A-rings in flavan-3-ol monomers cross-link with formaldehyde to create a strong adhesive polymer (Pizzi, 1980). These tannins, also known as proanthocyanidins (PACs), are composed of flavan-3-ol monomers connected by C-C bonds, forming various oligomer structures (African Territories Wattle Industry Fund Limited, 1980)), as illustrated in Figure 1.4.



Fisetinidol $R_1 = R_2 = H$ (274 Da)
Catechin $R_1 = OH$, $R_2 = H$ (290 Da)
Robinetinidol $R_1 = H$, $R_2 = OH$ (290 Da)
Gallocatechin $R_1 = R_2 = OH$ (306Da)

Figure 1.4 The four flavan-3-ol monomers that are the basis of condensed tannins in black wattle bark (Source: Venter *et al.,* 2012).

The four flavan-3-ol monomers foundational to condensed tannins in black wattle bark are, (-)-fisetinidol, (+)-catechin, (-)-robinetinidol and (+)-gallocatechin (Venter *et al.,* 2012). Unlike hydrolysable tannins, condensed tannins produce a red colour when heated with aqueous acid, which is a critical factor affecting the market value

of the extract powder, with darker extracts considered inferior (Tindale and Roux, 1969; Falcão and Araújo, 2018).

The molecular weight of tannins, typically ranging from 1200 to 1300 Dalton (Da) in wattle extract, influences their penetration into hides during tanning (Hagerman, 2002). This relatively large molecular size, combined with lyophilic functional groups, enhances the solubility of tannins, a crucial property for their application in leather tanning (Evelyn, 1957).

Black wattle bark extract is among the purest commercial vegetable tanning materials, with low levels of acids and salts, making it ideal for tanning all types of leather (Shuttleworth and Cunningham, 1948). However, these condensed tannins are unstable and darken when exposed to sunlight and heat, posing challenges for maintaining extract quality (Hills, 1997).

1.5.2 Composition of Wattle Bark

Wattle bark is primarily composed of cellulose, lignin, and polyphenolic tannins. Hot water extraction removes tannins from the raw bark, also extracting non-tannin constituents. The three key components influencing wattle extract production are soluble tannins, non-tannins, and insoluble materials (Roux *et al.,* 1975).

Non-tannins, including low molecular mass flavonoids, sugars, and mineral salts, do not strongly bind to proteins (Reid *et al.,* 2013). According to an old Wattle Research Institute (WRI) report (1956-1957), the non-tannin portion of wattle bark extract comprises monosaccharides (fructose, glucose, and (+) Pinitol), disaccharides (such as sucrose), and polysaccharides. It also includes hydrocolloid gums (6-12%) and nitrogen-containing compounds (3%), which encompass amino acids like (-)-L-Pipecolic acid, (-)-L-4-hydroxy-trans-pipecolic acid, and L-proline, along with smaller quantities of amino acids (Auad *et al.,* 2020 ; Churms and Stephen,1991).

Tannin content in mature black wattle bark ranges from 27.1 % to 41.8 %, with an average of 36.8 % on a moisture-free basis (Sherry, 1971). Tannin levels vary across different parts of the tree as listed in Table 1.1, with the highest concentrations found in the bark.

Table 1.1. The distribution of tannins and their associated non-tannins in black wattle trees (Gujrathi and Babu,2007).

Part of the Tree	% Tannin	% Non-Tannin	% Insoluble	% Moisture
Bark (dry)	38.6	10.3	43.0	8.1
Leaves only	4.9	8.4	44.8	41.9
Leafless twigs	3.6	5.9	44.6	45.9
Upper stem	0.4	6.7	92.9	-
Stem base	1.6	3.3	95.1	-
Roots	12.7	13.5	73.1	10.0
Pods	21.6	13.5	51.8	13.1

Tannin content usually increases with increasing tree age (maturity at eight to ten years) and bark thickness (Dunlop, 1998).

1.5.3 Tannin Colour

Freshly stripped, mature bark is preferred for producing lighter-coloured extracts, which are used in various black wattle extract powders and solid extracts. Darker, weathered bark is used in adhesives where colour is less critical (Havemann, 1992). Mimosa ME extract powder, a natural product without chemical additives, is often blended with other vegetable tanning materials and synthetic tannins for tanning various types of leather. Light-coloured spray-dried powders like Mimosa GS, FS, and MS are used to achieve the lightest shades in finished leather without compromising tanning properties (Dunlop and MacLennan, 2002).

Tannins develop red and blue tints when exposed to ultraviolet light, heat, or mineral acids due to the hydroxylation of ring structures (Tindale and Roux, 1969). Environmental factors such as hot, moist conditions during harvesting and transport, as well as exposure to UV light, can further degrade the colour of bark extracts (Havemann, 1992; Sherry, 1971). Degraded bark extracts can be treated with sodium metabisulfite to lighten their colour, although this treatment alters their tanning applications (Hillis, 1997).

1.5.4 Analysis of Tannin and Colour

Industry-standard methods, like the Society for Leather Technologists and Chemists (SLTC) or hide powder method, are used to analyse tannins in wattle bark extracts. These methods simulate the leather tanning process and provide benchmarks for determining non-tannins, insoluble content, moisture, and colour (Havemann, 1992). The tannin content, expressed as a percentage of the original sample, is measured by the quantity of tannin bound to the hide powder.

While alternative methods, such as the Stiasny method for adhesives (Pizzi, 1980) and ultraviolet spectroscopic analysis (Auad *et al.,* 2020), have been developed, they have not yet replaced the SLTC method for commercial transactions. Techniques like near-infrared reflectance spectroscopy (NIRS) and nuclear magnetic resonance (NMR) spectroscopy are also used to estimate tannin content (Siesler *et al.,* 2008). Additionally, pyrolysis/gas chromatography (GC), electrospray mass spectrometry (EMS), and quantitative NMR analyses have been explored for tannin content estimation (Yazaki *et al.,* 1993).

NIRS has shown potential as a rapid, low-cost method for estimating tannin and non-tannin yields in bark extracts, with accuracy comparable to or better than the hide powder method (Donkin and Pearce, 1995; Schimleck and Workman, 2004). Although NIRS has not been previously used for colour determination, it could be employed to predict various quality aspects of wattle bark, ranging from cultivar identification to extract powder analysis.

The colour of tannin extract powders is measured using the Lovibond Tintometer, where a tannin solution is visually matched with red and yellow filters to determine its colour. This method, though standard in the leather industry, is subjective and may suffer from variation in reproducibility (Garbutt and Nobel, 1983).

1.5.5 Bark Analysis

The factory method for analysing tannin content involves reflux extraction of the bark, followed by the determination of extractives, non-tannins, and soluble percentages. These methods, while effective for extract analysis, may not accurately determine the intrinsic colour of the bark extract due to the darkening of chemical constituents during the extraction process (Beard, 1957).

1.6 Factors Influencing the quality of black wattle bark and bark extract

Field evaluations of bark properties have highlighted various factors affecting bark quality. A study by Nicholson (1991) examined bark samples from trees aged 7 to 17 years across KwaZulu-Natal, Mpumalanga, and the Cape. The study identified correlations between the amount of extractives in the bark and factors such as proximity to the sea, altitude, rainfall, and soil composition. However, even the most significant factor only explained 20 % of the variance in extractives (Nicholson, 1991).

1.6.1 Wattle Silviculture

Wattle plantations are established either through natural regeneration from seeds in the soil or by planting seedlings. Natural regeneration can lead to inferior plantations with uneven tree spacing, requiring labour-intensive thinning to produce quality plantations (Widrlechner, 1998). Seedling planting, now used for over 90 % of plantations, offers improved tree quality due to genetic enhancements (Chan and Isik, 2019). Clonal seedlings, though more expensive, provide uniform growth and consistent properties, making them the preferred choice in countries like Brazil (Stein and Tonietto, 1997). Advances in silviculture and genetics are vital for improving bark quality and the sustainability of the wattle industry (NCT Forestry Agricultural Co-operative Limited (NCT) News and Views, 2018).

1.6.2 Bark Harvesting and Delivery

Black wattle bark is processed into powdered and solid extract products at factories in KwaZulu-Natal and Mpumalanga. Harvesting occurs from spring to late autumn, as frost and dry winter conditions make bark stripping difficult. Adequate rainfall at the start of spring is essential for successful harvesting operations (Beard, 1957).

The quality of wattle bark depends on how quickly it is delivered to the factory after harvesting. Delays in transportation can lead to bark degradation due to oxidation and fermentation, resulting in lower-quality, darker extracts that fetch lower market prices (Beard, 1957). Efficient logistics are essential to maintain bark quality and ensure the sustainability of the industry.

1.6.3 Bark Stripping

The optimal felling age for black wattle trees is between 8 to 10 years, when the bark is thickest and yields the highest-quality extract (Sherry, 1971).

1.6.3.1 Manual Bark Stripping

In manual stripping, trees are felled and debranched, then cut into 2.4-meter lengths for easier handling. Bark is stripped using a hatchet, with strips bundled and transported to the factory within 24 hours to maintain quality. The average yield is about 15 tons of fresh bark per hectare (Havemann, 1992). The presentation and cleanliness of bark bundles significantly influence grading at the factory.

1.6.3.2 Mechanical Bark Stripping

Manual stripping is favoured in South Africa for its employment benefits and precision in the removal of the tannin-rich outer bark, preserving its integrity and minimising contamination with low-tannin materials (Mackenzie,1962). However, research into mechanical debarking has found it to more efficient and safer, although it results in reductions in tannin content (SA Forestry Online, 2009). Mechanical stripping decreases tannin content by removing tannin-rich outer bark layers, incorporating low-tannin materials, generating heat and friction that degrade tannins, causing uneven stripping, fragmenting bark that accelerates oxidation, and damaging cell structures, leading to reduced extractable tannins (Haojie, 1997). However, Eggers found that mechanical debarking could be beneficial during cold months when manual stripping is less feasible (Eggers, 2010).

1.6.4 Bark Quality and Grading Practices

Bark quality criteria have remained consistent since the mid-20th century. At factories, bark is weighed and visually assessed, with grading based on presentation, freshness, and quality. The best-quality bark is neatly bundled, with an ideal thickness of 5 mm and minimal discolouration, this is illustrated in Figure 1.5 (A) and (B). Proper grading practices are essential for optimizing extract production and ensuring the quality of the final product.

Figure 1.5 (A) Fresh black wattle bark illustrating a pale white cambium and (B) the ideal presentation of bark strips in bundles (Source: Janse van Rensburg, 2022).

To account for varying transport times to factories or depots, three bark grade levels have been established (Kassier, 1959):

- **Fresh grade**: 0-24 hours, no visible oxidation.
- **Average grade**: 24-48 hours, minimal oxidation.
- **Merch grade**: Over 48 hours, moderate to high oxidation.

Inferior-quality bark, which significantly impacts the quality of the final extract, is rejected by depots and factories. Figure 1.6 (A -E) illustrates examples of such inferior-quality bark.

Figure 1.6 The several types of bark that are rejected due to inadequate quality as illustrated above (A) immature bark, (B) corky bark, (C) moldy bark, (D) green wattle, (E) poor presentation, and (F) dry bark (Source: Janse van Rensburg, 2022).

Factory monitoring of quality parameters, including tannin content, extract colour and bark extraction efficiency, is crucial. While bark grade significantly influences these parameters, other factors such as the season of bark stripping (spring, summer, autumn) and the handling conditions of the commercial powder also impact quality. These factors are detailed in Table 1.2.

Table 1.2 The list of factors affecting tannin colour change in wattle bark and wattle extract powder.

Material	Factor
Wattle bark	- Environmental conditions (rainfall, temperature, etc)
	- Disease
	- Handling and delivery time to the factory
	- Inferior clones produced under breeding programmes
	- Materials and impurities causing contamination (e.g. Iron contamination)
	- Green wattle bark contamination
Black wattle extract	- Extraction process/ conditions (Heat, pH of process streams, processing times, and aeration)
	- Chemicals
	- Oxidation after spray drying
	- Storage

1.6.5 Stick vs. Fresh Bark

Historically, South Africa exported dried wattle bark, known as "stick bark," for processing abroad. This practice declined with the development of domestic wattle extract factories (Mackenzie, 1962). "Stick bark," air-dried to reduce moisture content, is still produced when factory capacity is limited or during the off-season. When dried properly, “stick bark” can produce a high-quality tannin extract. The tannin content is often higher due to the reduced moisture content in the bark, as well as the oxidation of tannins, which increases their concentration. However, the extract derived from “stick bark” typically has a darker colour (Gujrathi and Babu, 2007). The colour properties of stick bark are less extensively documented. Fresh

bark remains the preferred choice for processing due to its superior tannin quality, paler colour and less processing steps in comparison to stick bark (Hillis, 1997). Exploring ways to improve "stick bark" quality could enhance tannin production during peak harvest periods when processing capacity is exceeded.

1.6.6 Factory Processing and Bark Extraction

Wattle bark undergoes a detailed extraction process at the factory. After grading, bark strips are chopped into uniform 6 x 6 mm pieces to maximize tannin extraction. Approximately 3.33 tons of fresh bark chips, with 50 % moisture content, produce one ton of wattle bark extract powder. The extraction occurs in autoclaves using a counter-current process with hot water at temperatures between 95 °C and 120 °C, depending on the product specification (Gujrathi and Babu, 2007).

The initial extract, or "thin liquor," contains 7-9 % solids and is concentrated to "thick liquor" containing approximately 50 % solids. At this stage, the factory will determine which product to manufacture. The "thick liquor" can be spray-dried to create a natural vegetable extract, such as Mimosa ME powder, or treated with various chemicals to produce vegetable tannin powders with altered properties, like Mimosa GS (bleached), Mimosa FS, and others. The final product has approximately 6 % moisture and is packaged for export. Alternatively, the "thick liquor" can be further concentrated to around 16 % moisture to produce Mimosa Solid, a toffee-like substance that hardens upon drying. Products sensitive to light or heat are stored in cool, dark conditions to prevent deterioration. The entire process, from bark stripping to extract powder, is shown in Figure 1.7.

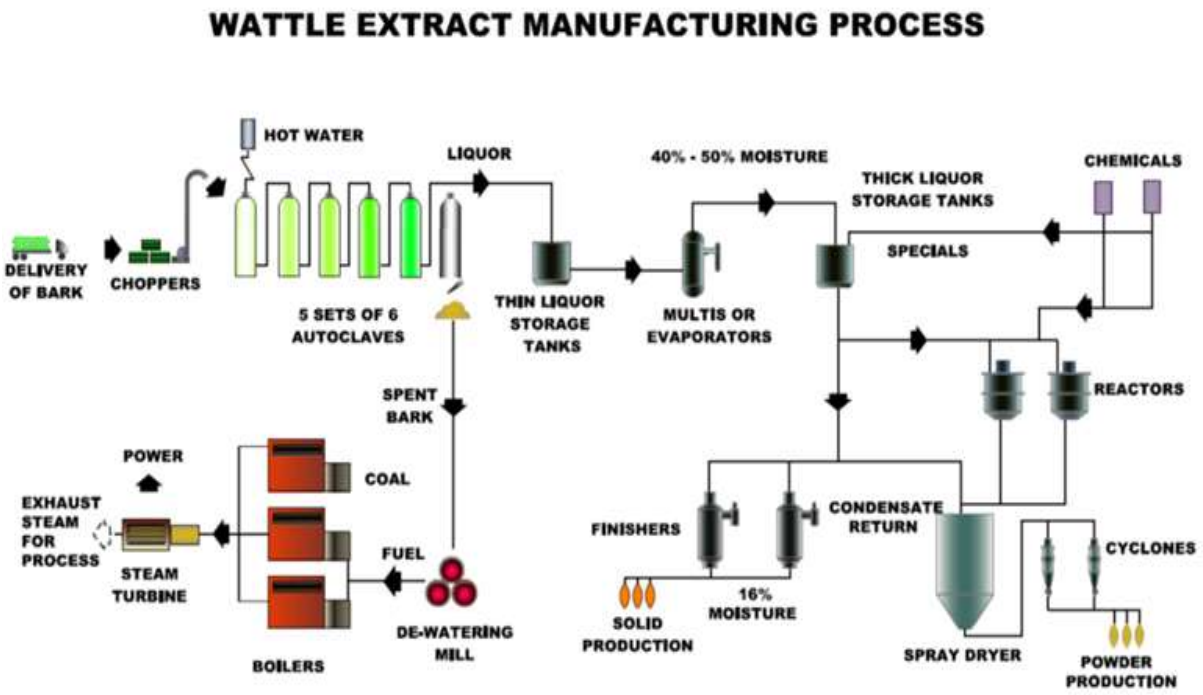


Figure 1.7 The wattle bark extract manufacturing process (Source: NTE Company, 2022).

The bark extraction process generates spent bark as a byproduct. After dewatering to reduce moisture, the spent bark is burned in a multi-fuel boiler to produce steam for the extraction process. It can also be repurposed for fertilizer production (Gujrathi and Babu, 2007).

1.6.7 Process monitoring and quality control- bark and liquor analysis

Process monitoring and quality control are essential during factory bark extraction. Samples of chopped bark and extraction liquors are analysed in quality control labs for fibre, total extractives, tannins, non-tannins, insoluble content, and moisture. These analyses determine factory efficiency and product quality, using key metrics such as the extraction ratio (ER) calculated by dividing the tons of raw material by the amount of product manufactured and the index of quality (IQ). The IQ assesses the purity of the product and is calculated by dividing the extractives by the fibre of the bark.

Thin and thick liquors are evaluated for solids content using a halogen moisture analyser (HMA). The thick liquor is further analysed for density and colour using a

densitometer and a Lovibond Tintometer, respectively. Colour analysis, based on SLTC parameters, helps determine the final product's suitability by assessing its redness and yellowness (Bhagwandin, 2022).

The tannin-to-non-tannin ratio (T/NT) is a critical quality measure for black wattle bark, ideally above 2.6. This ratio is significantly influenced by the amount and frequency of rainfall during harvest, as well as atmospheric humidity (Elmer *et al.,* 1964).

1.6.8 Genetic Factors

Bark quality in black wattle trees vary significantly due to genetic diversity, plantation site, and environmental stress. Research indicates that enhancing black wattle through breeding techniques such as provenance testing, vegetative propagation, and progeny testing can improve bark quality (Sewpersad, 2004). Variations in tree characteristics, including colour properties of tannins, can be optimised through clonal breeding and the establishment of seed orchards. While genetic uniformity in clonal forestry poses some risks, the benefits of consistent raw materials and enhanced tannin production outweigh the disadvantages (Dunlop *et al.,* 2003).

1.6.9 Green Wattle

Green wattle (*Acacia decurrens*), though frost-tolerant and resistant to rust (Glueck, 1952), produces bark with a darker extract that does not meet the quality standards required by leather tanners (Tindale and Roux, 1969). Despite its superior growth under adverse conditions, the South African government restricted its planting in favour of black wattle, which offers better-quality bark for the tannin industry (Craib, 1941).

1.6.10 Tree Age

Tree age significantly influences bark tannin content, which stabilizes as trees mature. A study conducted at the ICFR across ten farms (spanning six different locations) and five age classes demonstrated that the ideal age for felling black wattle ranges between seven and eleven years (Dunlop, 1998). Table 1.3 summarizes the average data collected during this trial.

Table1.3 Average tannin content over different tree age classes (Dunlop, 1998)

	Average tannin content per tree age class				
	Age 7	**Age 8**	**Age 9**	**Age 10**	**Age 11**
Farm 1	38.5	39.0	39.5	41.0	39.5
Farm 2	42.0	42.5	43.0	44.0	39.0
Farm 3	N/A	38.0	41.0	36.0	N/A
Farm 4	38.0	39.0	40.0	42.0	44.0
Farm 5	42.0	38.0	37.0	38.0	39.0
Farm 6	40.0	38.0	38.0	39.0	N/A
Farm 7	39.0	39.5	38.0	N/A	N/A
Farm 8	38.0	38.0	37.0	N/A	N/A
Farm 9	N/A	39.0	39.5	40.0	40.0
Farm 10	N/A	37.0	36.5	36.0	N/A
Average	39.6	38.8	39.0	39.5	40.3

Arguably based on the averages of this study, 7 years would be the ideal age, however the 10-year age class is favoured over 7 years because it yields higher tannin content and greater bark volume. At 10 years, the trees produce more mature and marketable timber suitable for multiple applications, whereas at 7-year-old trees have thinner bark and lower timber quality. Eleven-year-old trees were not considered as the bark is much thicker and harder to strip. In Brazil, trees are typically felled from seven years onward, balancing demand for both timber and bark. Younger trees may provide softer, more strippable bark; however, this bark is much thinner. Understanding the precise impact of tree age on bark quality is crucial for optimizing harvest techniques (Stein and Tonietto, 1997).

1.6.11 Environmental Conditions

Environmental factors, particularly frost, significantly impact black wattle bark quality. Frost damage, especially in the first four months of growth, can kill young trees and reduce bark supply (Chan, 2019). Other environmental influences include climate, rainfall, and soil moisture, all of which affect bark moisture levels and extractive content. Low moisture at harvest correlates with lower extractive concentrations and diminished bark quality. These factors highlight the importance of understanding environmental conditions to optimise bark harvesting and processing (Elmer *et al.,* 1964).

Figure 1.8 illustrates a downward trend of bark moisture, which suggests a decline in extractives and overall extract quality during periods of dryness (drought).

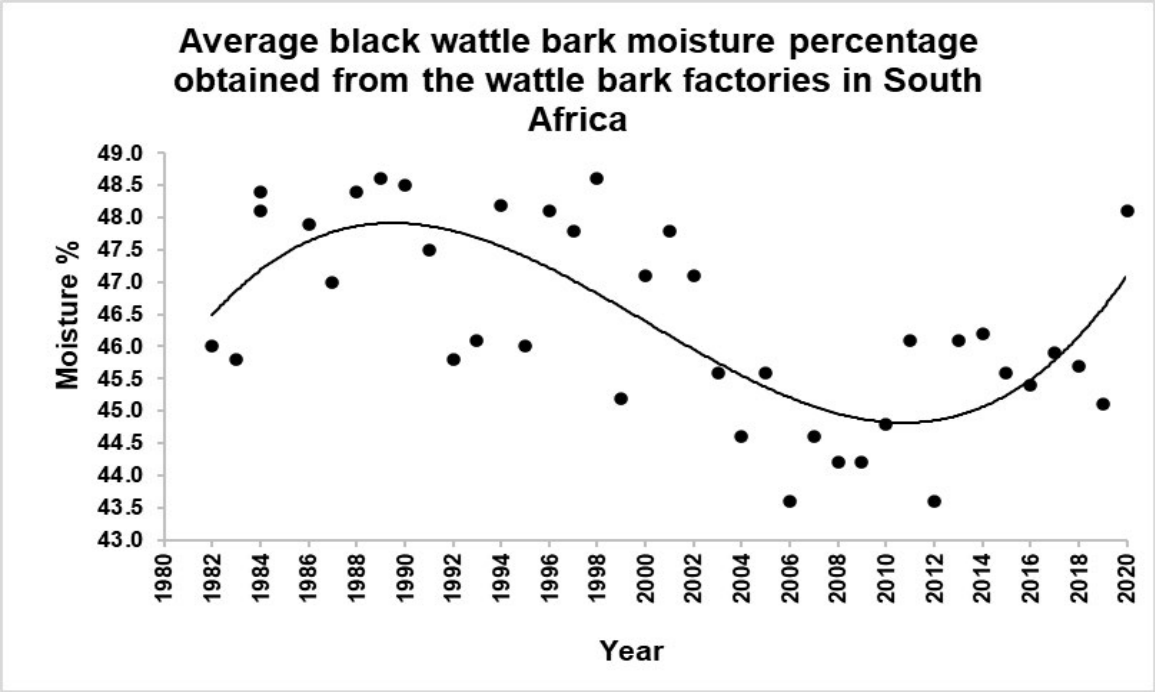


Figure 1.8 Average moisture percentage of fresh black wattle bark from wattle bark factories in South Africa (NTE and UCL data from 1982 until 2020). The solid-fitted trendline shows the general changes over time.

A technical report on the climatic effect on factory wattle extract quality was conducted at the ICFR. A significant finding of the report was that rainfall is the primary factor affecting tannin content and colour quality in wattle bark. Temperature's impact on bark quality is also influenced by rainfall, while other factors such as site quality and genetics play significant roles, warranting further research. Climate change poses a considerable risk to the tannin industry, particularly with the potential for longer droughts and extreme rainfall. Mitigation strategies may include better site-species matching and breeding for drought tolerance, focusing on maintaining adequate bark moisture levels (Burgdorf and Germishuizen, 2021).

1.6.12 Pests and Diseases

Pests and diseases, such as wattle rust and "Blackbutt" disease caused by *Phytophthora nicotianae* var. *parasitica*, significantly impact bark quality and yield. Wattle rust, identified in 2013, defoliates trees and has spread across all wattle-growing regions in South Africa, leading to reduced tannin quality and increased

mortality (Oumar, 2020). Diseases like "Blackbutt" cause severe bark degradation, affecting both yield and quality, with diseased bark often producing darker extracts (Gordon-Gray, 1965).

High tree mortality which affects bark production is also influenced by soil invertebrate pests such as white grubs (larvae of Coleoptera (*Anommatocoris coleopteratus* Kormilev)) and cutworms (larvae of lepidoptera (*Coryssocnemis lepidoptera* Mello-Leitão*)),* and also millipedes, nematodes, grasshoppers, ants, false wireworms, termites and crickets (Govender, 2014). The interaction between these pests, diseases, and tannin quality warrants further investigation.

1.6.13 Soil Properties and Bark Quality

Black wattle thrives in deep, moist, fertile soils but can also grow in soils with a pH range of 4.5 to 7.0 (Booth, 1997). Soil quality directly impacts timber, bark yield, and bark quality, with issues such as increased gummosis prevalent in poor soil conditions. While fertilizer application can enhance yield, it may also increase gummosis, negatively affecting bark quality (Schonau, 1970). Phosphorus (P) and potassium (K) fertilizers have been shown to improve yields, whereas lime reduces bark quality (Titshall, 2021).

1.7 Discussion

A study conducted in 1927 at Cedara, later published in 1934, provided a detailed examination of various factors influencing colour quality, several of which are proposed in this review as potential areas for further research. This comprehensive investigation considered multiple variables, including tree age, site quality, and geographic location. The findings from this study offer a valuable foundation for future research, particularly in understanding how these factors impact tannin content and colour quality (Williams, 1934).

From the late 1940s to the 1990s, significant research was conducted on black wattle at the Wattle Research Institute (WRI) and the Leather Industries Research Institute (LIRI) in South Africa. This research focused on improving the tannin content of wattle bark and its applications in leather tanning. However, the colour properties of wattle bark extracts received limited attention and have not been significantly explored since the closure of LIRI in 2000 and the shift in focus of the

WRI to broader forestry research under the Institute for Commercial Forestry Research (ICFR) (ICFR, 2017).

The breeding programs of the 1950s prioritised bark quality, particularly tannin content. However, after 2002, the focus shifted towards improving timber yields as timber became more economically valuable than bark. Recent breeding efforts aim to enhance timber yields, increase resistance to pests and diseases, and maintain or improve bark quality. Continuous monitoring and selective breeding are crucial to ensuring progress in these areas (Dunlop and MacLennan, 2002).

Despite the decline in research on wattle extract quality in recent years, understanding tannin quality remains essential. Modern technologies like near-infrared spectroscopy (NIRS) can facilitate high-volume, low-cost screening and monitoring of tannin composition, supporting efforts to maintain and improve extract quality.

1.8 Future Prospects for the Wattle Bark Extract Industry

As land dedicated to wattle cultivation decreases, the industry's future depends on developing alternative uses for wattle extracts and preserving or expanding existing cultivation areas (Chan *et al.,* 2015). Emerging applications for wattle tannins, such as renewable antioxidants in biodiesel production and nutraceuticals, offer promising avenues for diversification and growth. These innovations could enhance the long-term sustainability and viability of the wattle bark extract industry (Schaumlöffel *et al.,* 2021; Singh and Kumar, 2020).

To further improve the industry, a comprehensive approach to silviculture and harvesting practices is needed. Identifying critical stages where bark colour darkening occurs and implementing mitigation strategies such as applying reducing agents, cold-chain storage, and protective packaging could help maintain quality during transport and processing (Cheng and Hagiopol, 2021).

The black wattle industry has a deep-rooted history in South Africa and serves as a significant player in the global leather tanning and extract markets. Understanding its historical background, production processes, challenges, and opportunities is vital for fostering sustainable growth and competitiveness. By addressing environmental

issues, embracing sustainability practices, and exploring novel product avenues, the industry can solidify and expand its position in the dynamic global market.

1.9 References

African Territories Wattle Industry Fund Limited. 1980. The Properties, Composition, Reactions and Industrial Applications of Mimosa Extract. Hanbury, Tomsett, and Co. Ltd. London. Pp 8 -14.

Antoine, M.L., Simon, C. and Pizzi, A. 2004. UV spectrophotometric method for polyphenolic tannin analysis. *Journal of Applied Polymer Science*, *91*(4), 2729-2732.

Auad, P., Spier, F. and Gutterres, M. 2020. Vegetable tannin composition and its association with the leather tanning effect. *Chemical Engineering Communications*, *207*(5), 722-732.

Beard, J.S. 1957. A Manual for Growing Wattle. Natal Tanning Extract Company Limited, Pietermaritzburg. Pp1-11.

Bhagwandin, A. 2022. Personal communication with Mr. A. Bhagwandin, Production Chemist, NTE Hermannsburg, Hermannsburg, January 2022.

Booth, T.H. 1997. Climatic Factors. In Brown, A.G., and Ko, H.C. (Eds): *Black Wattle and Its Utilisation*. Rural Industries Research and Development Corporation, Canberra. Pp 13 -18.

Burgdorf, R., and Germishuizen, I. 2021. Climatic effects on factory Mimosa extract quality. *Institute for Commercial Forestry Research* (Unpublished).

Campbell, PL, Naude, DC & Kluge, R.L.1999. Development of integrated control strategies for the wattle. II. Control of unwanted wattle. *South African Journal of Plant and Soil*, *16*(1),31-37.

Chan, J.M., Day, P., Feely, J., Thompson, R., Little, K.M. and Norris, C.H. 2015. *Acacia mearnsii* industry overview: current status, key research, and development issues. *Southern Forests: A Journal of Forest Science*, *77*(1), 19-30.

Chan, J.M. and Isik, F. 2019. Frost tolerance of six seed orchards of *Acacia mearnsii* (black wattle) and the effect of developmental stage and tree size on frost hardiness. *Australian Forestry*, *82*(1), 35-47.

Chaudhary, A., and Prasad, E. 2021. *Tannin Market. Global Opportunity Analysis and Industry Forecast, 2021-2030.* Allied Market Research, United States. Tannin Market Size, Share | Industry Growth & Forecast, 2030 (alliedmarketresearch.com). Accessed: April 2022.

Chaunbi, G. 1997. Black wattle plantations in South Africa: Introduction, Silviculture, and Management. In Brown, A.G., and Ko, H.C. (Eds): *Black Wattle and its Utilisation*. Pp 28 - 42.

Cheng, K. and Hagiopol, C. 2021. Wood tannins: structure, production, and analysis. *Natura -I Polyphenols from Wood: Tannin and Lignin–An Industrial Perspective*, pp 85 -96.

Clarke, J. 2018. Job creation in agriculture, forestry, and fisheries in South Africa: *An analysis of employment trends, opportunities, and constraints in forestry and wood products industries*. University of Western Cape publication. Pp 20-36.

Craib, I.J. 1941. South African wattle bark and wattle extract with special reference to the American market. *Journal of the South African Forestry Association*, *6*(1), pp.71-88.

Churms, S.C. and Stephen, A.M., 1991. Chromatographic separation and examination of carbohydrate and phenolic components of the non-tannin fraction of black wattle (*Acacia mearnsii*) bark extract. *Journal of Chromatography A*, *550*, pp.519-537.

Donkin, M.J. and Pearce, J. 1995. Tannin analysis by near-infrared spectroscopy. *Journal of the Society of Leather Technologists and Chemists*, *79*(1), 8-11.

Dunlop, R., 1998. *Final report on two Australian Acacia mearnsii (black wattle) provenance trials established in KwaZulu-Natal and south-eastern Mpumalanga.* ICFR Bulletin Number 7/98. Institute for Commercial Forestry Research. Pp 41-45.

Dunlop, R.W. and MacLennan, L.A. 2002. Chapter 1: Introduction, Black *Wattle: The South African Research Experience*. Institute for Commercial Forestry Research. Pp 1-5.

Dunlop, R.W., Barnes, R.D., Nixon, K.M. and Hagedorn, S.F., 2003. A breeding strategy for *Acacia mearnsii* (black wattle) in South Africa: management paper. *Southern African Forestry Journal*, *2003*(199), pp.55-63.

Dyer, C. 2014. New names for the African *Acacia* species in *Vachellia* and *Senegalia*. *Southern Forests: A Journal of Forest Science*, *76*(4), iii-iii.

Eggers, J.R., 2010. *Acacia mearnsii debarking comparing different debarking technologies in the KwaZulu-Natal and Mpumalanga forestry regions of South Africa*. Ph.D. thesis, Nelson Mandela Metropolitan University. Pp 80-114.

Elmer, J.L., Gosnell, J.M. and Smith, R.G. 1964. A consideration of factors affecting quality in wattle bark. *East African Agricultural and Forestry Journal*, *29*(4), 350-361.

Evelyn, S.R., 1957. *Some Physico-chemical Aspects of Black Wattle Tannins with Special Reference to Molecular Weight*. Ph.D. thesis, University of South Africa, Pretoria. Pp 53-63.

Falcão, L. and Araújo, M.E.M. 2018. Vegetable tannins are used in the manufacture of historic leathers. *Molecules*, *23*(5),1081.

Forestry South Africa. FSA reports 1910 – 2021.

Garbutt, D.C.F. and Noble, M.A. 1983. *Diethylaminoethyl Cellulose as a Reagent in Tannin Analysis.* Report-Wattle Research Institute. Pp 115-120.

Glueck, L.D. 1952. *A Preliminary Investigation of the Structure of Green Wattle Tannin (Acacia decurrens Wild).* Ph.D. thesis, Rhodes University. Pp 1-10.

Gordon-Gray, C.G. 1965. The development of bark sampling from trees and plots in wattle plantations. *Wattle Research Institute.* Pietermaritzburg. Pp 36-39.

Govender, P. 2014. Effects of plantation residue management on the community structure of wattle regeneration invertebrate pests in South Africa. *Southern Forests: A Journal of Forest Science*, *76*(4), 229-236.

Gujrathi, A.M. and Babu, B.V. 2007. Environment-friendly products from black wattle. *Energy Education Science and Technology*, *19*(1), 37-44.

Hagerman, A.E., 2002. The Tannin Handbook. The department of Chemistry and Biochemistry. Miami University. Oxford. Pp 1-6.

SA Forestry Online. 2009. Harvesting wattle bark the UCL way. Accessed February 2024.

Haslam, E. 1989. Plant Polyphenols: Vegetable tannins revisited. *Chemistry and Pharmacology of Natural Products.* Cambridge University Press. New York. Pp 3 -7.

Havemann, D.A.1992. *The Manufacture and Uses of Wattle Bark Extract.* Ph.D. thesis, University of Stellenbosch. Pp 17-48.

Hillis, W.E. 1997. Tannin chemistry. In Brown, A.G., and Ko, H.C. (Eds): *Black Wattle and its Utilisation*. Rural Industries Research and Development Corporation, Canberra, Australia. Pp*106*.

Haojie, W. 1997. Black wattle plantations in South Africa. In Brown, A.G., and Ko, H.C. (Eds): *Black Wattle and its Utilisation*. Rural Industries Research and Development Corporation, Canberra, Australia. Pp 39.

OEC World. https://oec.world/. Accessed: 22/03/2024.

ICFR Bulletin. 2017. Honouring the past, celebrating the present, and innovating the future. ICFR, Pietermaritzburg. Pp 1-4.

Janse van Rensburg, M. 2022. Personal communication with Mrs. M. Janse van Rensburg, Bark Procurement Officer. NTE Iswepe, Iswepe, January 2022.

Joslyn, M.A., and Goldstein, J.L. 1964. The astringency of fruits and fruit products in relation to phenolic content. *Advances in Food Research*, *13*, 179-217.

Kassier, W.E.1959. *An Economic Analysis of Wattle Farming in the Union of South Africa*. Ph.D. thesis, University of Natal. Pp 27-41.

Kenea, M. 2022. Assessing the main priorities area and opportunities for banks financing in leather industry in Ethiopia. *Research Journal of Finance and Accounting*, *13*(1), 2222-1697.

Khanbabaee, K. and Van Ree, T. 2001. Tannins: classification and definition. *Natural Product Reports*, *18*(6), 641-649.

Magona, N., Richardson, D.M., Le Roux, J.J., Kritzinger-Klopper, S., and Wilson, J.R. 2018. Even well-studied groups of alien species might be poorly inventoried: Australian Acacia species in South Africa as a case study. *NeoBiota*,39, 1-29.

Mackenzie, T.F. 1962. The wattle bark industry. *South African Forestry Journal*, *43*(1), 7-16.

Makkar, H.P.S. and Becker, K. 1998. Do tannins in leaves of trees and shrubs from African and Himalayan regions differ in level and activity? *Agroforestry Systems*, *40*(1), 59-68.

Morris, A.R. 2022. Changing use of species and hybrids in South African forest plantations. *Southern Forests: A Journal of Forest Science*, *84*(3), 193-205.

NCT News and Views. 2018. Number 94. December 2018. Pp 11-14. https://www.nctforest.com/

Nicholson, C.R.L. 1991. Re-appraisal of the variation of bark components and quality with age in black wattle trees. ICFR annual research report, Pietermaritzburg. Pp 221-225.

NTE Company 2022. *The Manufacturing of Black Wattle (Acacia mearnsii) Bark Extract (Bondtite 945) at NTE Company's Factories.* NTE Company (Pty) Ltd, Pietermaritzburg, South Africa. Pp 1-2

Ogawa, S. and Yazaki, Y. 2018. Tannins from *Acacia mearnsii* De Wild. Bark: Tannin determination and biological activities. *Molecules*, *23*(4), 837.

Oumar, S. 2020. *Assessing the Utility of Landsat 8 Multispectral Sensor and the MaxEnt Species Distribution Model to Monitor Uromycladium acaciae Damage in KwaZulu-Natal, South Africa*. Ph.D. thesis, University of KwaZulu-Natal. Pp 1-3.

Pinto, P.C., Sousa, G., Crispim, F., Silvestre, A.J. and Neto, C.P. 2013. *Eucalyptus globulus* bark is a source of tannin extracts for application in the leather industry. *ACS Sustainable Chemistry & Engineering*, *1*(8), 950-955.

Pizzi, A. 1980. Tannin-based adhesives. *Journal of Macromolecular Science—Reviews in Macromolecular Chemistry*, *18*(2), 247-315.

Pizzi, A. 2008. Tannins: major sources, properties, and applications. In *Monomers, polymers, and composites from renewable resources*. Elsevier. Pp 179-199.

Reid, D.G., Bonnet, S.L., Kemp, G. and van der Westhuizen, J.H. 2013. Analysis of commercial proanthocyanidins. Part 4: Solid state 13C NMR as a tool for in situ

analysis of proanthocyanidins tannins, in heartwood and bark of quebracho and acacia, and related species. *Phytochemistry*, *94*, 243-248.

Roux, D.G., Ferreira, D., Hundt, H.K. and Malan, E. 1975. Structure, stereochemistry, and reactivity of natural condensed tannins as the basis for their extended industrial application. In: *Applied Polymer Symposia.* 28, 335-353. Department of Chemistry. University of Orange Free State.

Schaumlöffel, L.S., Fontoura, L.A., Santos, S.J., Pontes, L.F. and Gutterres, M. 2021. Vegetable tannins-based additive as antioxidant for biodiesel. *Fuel*, *292*, 120-198

Schimleck, L.R. and Workman Jr, J. 2004. Analysis of timber and paper. *Near-Infrared Spectroscopy in Agriculture*, *44*, 633-646.

Schonau, A.P.G. 1970. The effect of fertilizer, lime, and trace element applications on growth and disease incidence in black wattle (*Acacia mearnsii de Wild*). Report. Wattle Research Institute, University of Natal, 1971.Pp 38-48.

Searle, S.D. 1997. *Acacia mearnsii* De Wild (black wattle) in Australia. In Brown, A.G., and Ko, H.C. (Eds): *Black Wattle and its Utilizations. Rural Industries Research and Development Corporation, Barton, ACT*. Pp 1-10.

Sewpersad, Y., 2004. *Estimation of genetic variation and marker identification in black wattle (Acacia mearnsii De Wild) with RAPD fingerprinting*. Ph.D. thesis, University of KwaZulu-Natal. Pp 15 -25.

Sherry, S.P. 1971. *The Black Wattle* (*Acacia mearnsii de Wild*). University of Natal Press, Pietermaritzburg. Pp 7-30 and 40-44.

Shuttleworth, S.G. and Cunningham, G.E. 1948. The theory of vegetable tannage. *Journal of the Society of Leather Trades' Chemists*, *32*(6), 183-209.

Siesler, H.W., Kawata, S., Heise, H.M. and Ozaki, Y. (Eds). 2008. *Near-infrared Spectroscopy: Principles, Instruments, Applications*. Wiley – VCH. Germany. Pp 179 – 213.

Singh, A.P. and Kumar, S., 2020. Applications of tannins in industry. In: *Tannins-structural properties, biological properties, and current knowledge*. London, United Kingdom. Pp 117-130.

Stein, P.P. and Tonietto, L. 1997. Black wattle silviculture in Brazil. *Black wattle and its Utilisation*.81 -85.

Stephens, R.P. 1940. Mining timber in South Africa with special reference to wattle silviculture. *Journal of the South African Forestry Association*, *4*(1), 55-74.

Stubbings, J.A. 1977. ACACIA. *South African Forestry Journal*, *102*(1), 8-14.

Tewari, D.D. 2001. Is commercial forestry sustainable in South Africa? The changing institutional and policy needs. *Forest Policy and Economics*, *2*(3-4), 333-353.

Tindale, M.D., and Roux, D.G. 1969. A phytochemical survey of the Australian species of *Acacia*. *Phytochemistry*, *8*(9), 1713-1727.

Titshall, L. 2021. Residual fertiliser and liming impacts in a long-term *Acacia mearnsii* trial. *Southern Forests: A Journal of Forest Science*, *83*(1), 88-96.

Venter, P.B., Senekal, N.D., Kemp, G., Amra-Jordaan, M., Khan, P., Bonnet, S.L. and van der Westhuizen, J.H. 2012. Analysis of commercial proanthocyanidins. Part 3: The chemical composition of wattle (*Acacia mearnsii*) bark extract. *Phytochemistry*, *83*, 153-167.

Wade, R. 2015. The role of industrial policy in developing countries. *Rethinking Development Strategies After the Financial Crisis*, *1*, 67-78.

Wattle Research Institute (WRI) Report. 1956/1957. Seasonal effects on extractives. Wattle Research Institute, Pietermaritzburg. Pp 26-27.

Widrlechner, M.P. 1998. Managerial tools for seed regeneration. In *Regeneration of Seed Crops and their Wild Relatives (Engles, JMM, and Ramanatha Rao, R. (Eds). Proceedings of a Consultation of a Meeting, 4~ 7 December 1995.* International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India pp133-139.

Williams, C.O. 1934. South African Tanning Materials (Part 1). The Black Wattle. Department of Agriculture (Cedara), Division of Chemistry, Vol. 84. Pretoria. South Africa.

Yazaki, Y., Gu, R., Lin, Y., Chen, W. and Nguyen, N. 1993. Analyses of black wattle (*Acacia mearnsii*) tannins: relationships among the hide-powder, the Stiasny, and the ultra-violet (UV) methods. *Holzforschung*, *47*(1), 57-61.

Chapter 2: A novel laboratory method for the extraction of black wattle (*Acacia mearnsii* de Wild.) bark constituents

P. Avadianund Bridglall, M.D. Laing, N. Naidoo, and R.J. Burgdorf

2.1 Abstract

Tannins are the major extractable component of black wattle (*Acacia Mearnsii* de Wild) bark. In a wattle factory, extracted tannin levels are one of several quality parameters (Extraction efficiency and insoluble content are some of the other parameters) that are monitored, typically using a modified Soxhlet/ reflux extraction method. However, this method is slow and laborious, and the extracted product is unsuitable for tannin color analysis, which is another important product quality parameter. A new method was then developed, aiming to measure both the conventional bark parameters as well as the color of the extract solution, and increase sample throughput. The effect of oven-drying versus freeze-drying was compared as a method of moisture removal for tannin color preservation prior to extraction. Sample filtration methods, extraction times, and chemical additives were also tested, compared, and optimised as necessary. Suitably processed bark samples were subjected to four extraction methods, i.e., Soxhlet, cold water, pressure cooker, and autoclave extractions. Extract properties were analysed using the standard Society for Leather Technologists and Chemists (SLTC) methods. Freeze-drying was shown to arrest color development in the bark. Deionised water was the best extractant tested, although the addition of ethylenediaminetetraacetic acid (EDTA) enhanced the color of the extracts. The autoclave extraction method, involving two extractions and centrifugation, was found to be the most practical and effective for wattle bark. It minimizes color change, reduces sample analysis variability, and allows for high sample throughput. This method is valuable for future research on wattle bark properties and for routine quality analysis in wattle extract factories.

Keywords: Bark, color, wattle, tannins, extractions

2.2 Introduction

Black wattle (*Acacia mearnsii* De Wild.) is cultivated commercially for its wood and bark. The wood is mainly processed into chips for export purposes, whereas the bark is utilized in factories to extract tannins (Chan *et al.,* 2015). Bark extract powder, known as Mimosa Extract, is a major export from the KwaZulu-Natal and Mpumalanga regions of South Africa (Chaunbi, 1997). The production of Mimosa Extract is a multi-stage process beginning with the cultivation of black wattle trees. Trees are felled at 8 -10 years old, in a period between early spring to autumn, when the bark is harvested.

At the factory, the bark extraction process uses large autoclaves in which hot, pressurised water is passed through numerous batches of chipped bark. The extracted liquor is concentrated by evaporation and spray-dried into a powder (Gujrathi and Babu, 2007). To ensure the highest quality tannin extracts, wattle bark must be as fresh as possible, as aging leads to tannin degradation. Aged bark produces darkened tannins, an undesirable trait that compromises both the visual appeal and functional performance of the final product. Darkened tannins can create uneven or blotchy finishes on leather, diminishing its aesthetic value and market appeal, particularly in fashion and luxury goods. Furthermore, the oxidation and degradation of these tannins reduce their tanning efficacy, weakening the cross-linking process with collagen in hides. This results in inferior leather that may become too stiff or susceptible to breakdown (Dunlop and MacLennan, 2002).

Quality control and research on tannin extraction have focused on optimal extraction efficiency and tannin content as the primary quality indicators. Chipped bark samples are routinely analysed at the laboratories of bark extraction factories. The standard method of laboratory analysis involves extraction by reflux, using equipment as shown in Figure 2.1. The reflux method adopted by factory laboratories is usually a modified version of this traditional Soxhlet method and is designed to mimic the action of the autoclaves in the factory for the extraction of tannins.

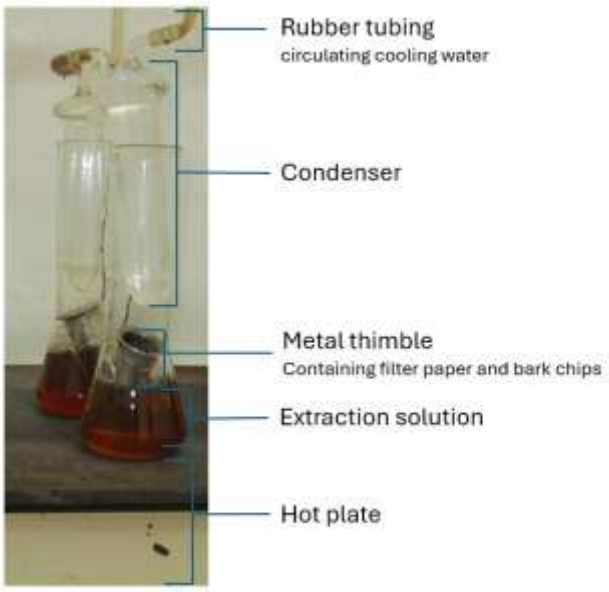


Figure 2.1 A reflux extraction of tannins from black wattle bark chips in a laboratory setting (Bhagwandin, 2022).

For the analysis, oven-dried bark chips are placed in a metal thimble lined with filter paper and boiled for several hours in deionised water, after which the solution is decanted and analysed for tannin content, total extractives, non-tannins, and insoluble solids using the Society for Leather Technologists and Chemists (SLTC) methods. No consideration is given to the extract solution color because the process aims to achieve maximum extraction of all water-soluble products. However, this causes severe darkening of the extract (Havemann, 1992) and prevents any assessment of bark tannin color quality. The extract darkens due to a chemical reaction driven by oxygen on the tannins as they are extracted from the bark (Hurlow, 1986).

Sherry's 1971 study reported tannin yields between 27.1 % and 41.8 %, averaging 36.8 % on a moisture-free basis, with site conditions influencing tannin content. This data guided the development of a high-throughput laboratory extraction method to enhance efficiency and color quality while maintaining typical tannin yields, addressing key factors affecting factory-level extract quality (Sherry, 1971).

The objectives of this study were to test various bark pre-processing and extraction methods with three primary goals:

1. To enhance speed of throughput

2. To preserve the extract color
3. To retain the efficient extraction of tannins, comparable with the current factory extraction process.

Bark pre-processing involved comparing freeze-drying to oven-drying because freeze-drying has been used to prevent color degradation and chemical changes in wattle bark tannins (Yazaki *et al.,* 1990). This study on extraction methods involved several sequential experiments that compared various extraction processes and parameters, including variation of temperature, equipment, filtration, time, and solvents.

2.3 Materials and method

2.3.1 Sample Collection

Black wattle bark samples were collected in September 2019 from the Harden Heights plantations (GPS coordinates 29.2667° S, 30.6167° E) in KwaZulu-Natal. Four ten-year-old trees were stripped at 1.3 m from the ground. The trees were ringed to remove strips of bark that were 10 cm high around the tree trunk. The mass of the stripped bark was between 200 to 300 g. The DBH (diameter at breast height) of the trees was between 12 - 15 cm. A cleaver was used to remove bark from the trees. The cleaver was stainless steel to minimize iron contamination that has been associated with tannin color changes (Slabbert, 1992). Samples were placed in plastic bags, vacuum-sealed, and placed in a cooler box with ice blocks to keep them cool. The samples were transported to the laboratory within two hours for further processing.

2.3.2 Comparison of drying methods

All samples were split into two portions. One portion was placed in a drying oven (Memmert, USA) at 60 ˚C for 48 hours until a constant mass was reached. The second group of samples was placed in a Virtis BT Pro series freeze dryer (United Scientific, South Africa) at -70 ˚C and 175 millitorrs (mTorr) for 48 hours when a constant mass was reached. Sample masses were recorded and the difference between fresh and dried mass was calculated. A visual comparison of freeze-dried and oven-dried samples was conducted to evaluate the change in color over the drying period. Moisture content was measured, and a paired t-test was conducted to

determine if there was a significant difference. The samples were then stored at -20˚C until further analysis.

2.3.3 Freeze-dried sample preparation

Bark samples were freeze-dried to a moisture content of less than 1 %. Four individual tree samples were combined to form one bulk sample. These were milled to 0.5 mm using a ZM200 Retsch mill (Verder Scientific, Germany). Each bulk sample was then split into four sub-samples to test for method consistency and to determine the level of experimental error.

2.3.4 Extraction of the bark

Milled, freeze-dried samples were used for all extractions because freeze–drying provided more consistency, and the bark powder was visibly lighter in color than the oven–dried bark powder. Samples were suspended in deionized water in a 1:100 mass (g) to volume (mL) ratio for extraction. For this experiment, 2.5 g sample of milled, freeze-dried bark was used and made up to a final volume of 250 mL dispersion. The samples were extracted using four apparatuses. Each apparatus provided a different extraction method, as described in methods 1 to 4 below:

Method 1: The Soxhlet method was performed using reflux of the sample with a vertical condenser, and a water inlet, as illustrated in Figure 2.2 below. However, a modification was made whereby milled, freeze–dried material could be used instead of oven-dried bark chips.

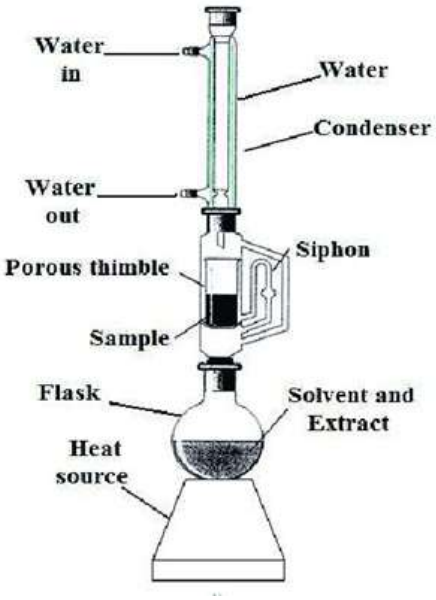


Figure 2.2 Soxhlet apparatus set-up used for the extraction of chemical components from solid material by reflux (Gupta *et al.,* 2012).

Method 2: The samples were extracted using a mechanical shaker (Model no: SPLMP8TUPF55 – Lab-Design Engineering, Labcon. South Africa) rotating at 60 revolutions per minute (rpm) for 16 hours at room temperature (±25 ˚C). This method was investigated as a low-temperature method of extracting the tannins to preserve bark tannin color properties.

Method 3: The samples were extracted using an electronic pressure cooker (AEG, Model – Precision EPC 6000, South Africa). The pressure cooker was set at the lowest temperature and pressure setting. The temperature and pressure of the pressure could not be determined as it was an automated domestic unit with no facilities for measurement of temperature or pressure.

Method 4: An autoclave (Speedy Autoclave, vertical type – Model No. HL -340) set at 100 ˚C and 100 kPa was used for the extractions. The laboratory autoclave was used to simulate the factory autoclave extraction.

Various combinations of extractant solvents, i.e., hot and cold water, water: ethanol mixture ratios, different filter papers and assorted extraction times were investigated. The flow diagram in Figure 2.3 is a summary of the methods and variables investigated.

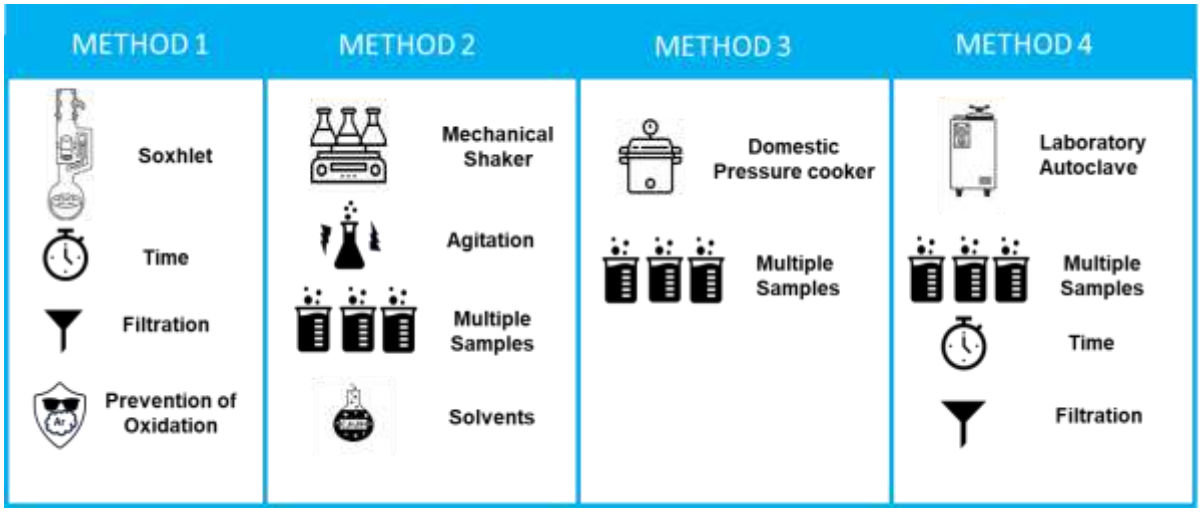


Figure 2.3 An illustration of the four methods and different variables investigated to optimise the bark extraction process.

The experiments are listed below, with variations of each step being examined as potential ways of improving the extraction method. A method of elimination was used to determine the best method. Treatments that showed favourable results were carried forward, and the less efficient methods were eliminated. The outline of each experiment is further detailed below from Experiments One to Nine.

Experiment One: Due to the control method taking an entire day for a single extraction, this experiment involved a modified version of the factory method with the intent to reduce the extraction times. This was done for two reasons, firstly to allow for a better sample throughput, and secondly, to preserve the color of the extracts. Table 2.1 describes the treatments.

Table 2.1 The testing of extraction time using the Soxhlet method for extraction of wattle bark.

Method	Apparatus	Solvent	Time (min) x Extractions	Filtration
[A] Control	Soxhlet	Hot Water	[B]180 x 2 120 x 1	Whatman 91
S1	Soxhlet	Hot Water	60 x 3	Whatman 91
S2	Soxhlet	Hot Water	30 x 3	Whatman 91
S3	Soxhlet	Hot Water	15 x 3	Whatman 91

Notes:

[A] The control method was used in Experiments One to Six and Experiment Eight for comparative purposes.

[B] The time for each extraction is in minutes and the number of extractions is indicated by the number, e.g., the control had 2 extractions of 180 mins and 1 extraction of 120 mins, whereas S1 had 3 extractions of 60 mins.

Experiment Two: Once a faster extraction process was identified, the method of filtration was investigated. Knowing that Whatman 91 filter paper is made up of cellulose, and that tannin binds to cellulose **(**Bae *et al.,* 1993), GF/C filter paper and no filter paper were compared to the control method. The milled freeze-dried bark was placed in the filter paper resembling a teabag for the extractions. For samples in which no filter paper was used, the samples were centrifuged (Centrifuge Uniscen, Orto Alresa, Spain) at 1500 rpm for three minutes after each extraction. The supernatants after each extraction were combined and made up to the final volume of 250 mL. Table 2.2 shows the treatments.

Table 2.2 The method set-up to compare the filtration process of wattle bark extract solutions using the Soxhlet method for extraction.

Method	Apparatus	Solvent	Time (min) x Extractions	Filtration
S1	Soxhlet	Hot Water	60 x 3	Whatman 91
S4	Soxhlet	Hot Water	60 x 3	GF/C
S5	Soxhlet	Hot Water	60 x 3	Centrifuged

Experiment Three: After the optimal method of filtration was determined, amber glassware and degassing of the solution were investigated as potential methods to improve the color of the extraction by reducing oxidation of the tannins whilst they were being extracted. Table 2.3 describes the treatments.

Table 2.3 The Soxhlet method of wattle bark extraction using amber glassware and Argon-degassed water.

Method	Apparatus	Solvent	Time (min) x Extractions	Filtration
S6	Soxhlet (Amber glassware)	Hot Water	60 x 3	Centrifuged
S7	Soxhlet	Hot Water (Degassed with Argon gas)	60 x 3	Centrifuged

Experiment Four: A mechanical shaker in combination with various solvents was investigated. The mechanical shaker allowed for multiple samples to be extracted at the same time, compared with the factory laboratory Soxhlet method, which can only extract from one sample at a time per glassware set-up. Amber glassware was tested to determine whether incident light would affect the color of the extract. The treatments used in this experiment are listed in Table 2.4.

Table 2.4 The mechanical shaker extract conditions for the extraction of wattle bark.

Method	Apparatus	Solvent	Time (min) x Extractions	Filtration
M1	Mechanical Shaker	Cold Water	960 x 1	Centrifuged
M2	Mechanical Shaker	15 % Water: Ethanol	960 x 1	Centrifuged
M3	Mechanical Shaker	85 % Water: Ethanol	960 x 1	Centrifuged
M4	Mechanical Shaker	Hot Water	960 x 1	Centrifuged

Experiment Five: A pressure cooker was used to extract multiple samples concurrently. The treatment is listed in Table 2.5.

Table 2.5 The pressure cooker treatment setup for the extraction of wattle bark.

Method	Apparatus	Solvent	Time (min) x Extractions	Filtration
P1	Pressure Cooker	Hot Water	60 x 3	Centrifuged

Experiment Six: This experiment involved the use of a laboratory autoclave. Extraction times were further investigated. The treatments are described in Table 2.6.

Table 2.6 The treatments of the autoclave method of wattle bark extraction.

Method	Apparatus	Solvent	Time (min) x Extractions	Filtration
A1	Autoclave	Hot Water	60 x 3	Centrifuged
A2	Autoclave	Hot Water	30 x 3	Centrifuged
A3	Autoclave	Hot Water	15 x 3	Centrifuged

Experiment Seven: This experiment was conducted to optimise the number of extraction processes applied to each sample.

Multiple extractions were done on milled freeze-dried bark to determine the number of extractions required to remove all the tannin from the bark. The samples were extracted using the autoclave method of extraction (Method A3). After each extraction was applied to each sample, an aliquot of the extract solution was taken. The aliquots were analysed on a UV Spectrophotometer (Genova Nano Spectrophotometer Jenway - Lasec, South Africa), with absorbance readings at 280nm (Yazaki *et al*, 1993). The absorbance values were correlated graphically to the amount of tannin extracted using linear regression in Microsoft Excel.

Experiment Eight: This experiment was conducted to determine the optimum time required for maximum tannin extraction.

The time of extractions was tested for both Soxhlet and autoclave extractions. This was compared to the control and Method A3 from Experiment Six. The treatments are outlined in Table 2.7 below.

Table 2.7 Comparing the efficiency of two and three extractions applied in the autoclave method of wattle bark extraction and compared to the Soxhlet method (control).

Method	Apparatus	Solvent	Time (min) x Extractions	Filtration
A3	Autoclave	Hot Water	15 x 3	Centrifuged
A4	Autoclave	Hot Water	15 x 2	Centrifuged

The extract solutions for each treatment were analysed by the SLTC method. A t-test was done in Microsoft Excel for each treatment against the standard extract method to compare the tannin content. The treatments that produced fewer desirable outcomes and differed significantly from the standard were not considered for further comparison.

Experiment Nine: This experiment aimed to identify the most effective filtration method. Among the methods evaluated, Method A4 demonstrated superior performance, combining speed with high sample throughput. Consequently, we further explored Method A4 to maximize tannin extraction and enhance the elimination of insoluble residues through a comparative analysis of different filtration techniques. Replicates of the same sample were extracted using Method A4. One batch of samples was centrifuged and made up to volume (250 mL) after two extractions. The second batch of samples was centrifuged and after the second extraction, the remaining solution was passed through an 80mm porcelain Buchner funnel covered with Whatman GF/C filter paper and glass wool (Merck, Germany). The extract was then made up to a final volume of 250 mL. A t-test was used to compare the tannin percentage and Lovibond tintometer color readings of the extracts generated by the two methods.

Methods not significantly lower than the Control ($p > 0.05$) were subjected to one-way ANOVA (*R* statistical programming (with the agricolae package)) to evaluate whether any alternative extraction procedures yielded equivalent or superior tannin

recovery relative to the Control. Significant ANOVA outcomes were followed by Fisher's LSD ((Cran, version 4.2.0)) mean separation to identify statistically comparable groupings, interpreted alongside Lovibond Red color to determine optimal extraction performance.

2.3.5 Analysis of bark extracts

The extracted solutions were analysed using the SLTC methods, i.e., extractives and tannin content analysis (Method SLT 2/3e), non-tannin content (Method SLT 2/3d), total soluble (Method SLT 2/3c), insoluble (Method SLT 2/3f), and color (Method SLT 2/3g), as described in the standard leather tanning SLTC methods (Leafe, 1999). The determination of each parameter is described below:

- The extractives and total soluble content are determined from the dry mass of the extraction solution.
- The insoluble content is determined by the difference between the extractives and the total soluble content of the extract solution.
- In the hide powder or SLTC method, the extract solution is added to animal hide powder (BLC Leather Technology, USA) that has been chemically treated with 3 % chromium alum or chromium (III) potassium sulphate solution (Merck, Germany).
- The tannins in the extract solution bind to the proteins in the animal hide powder. The non-tannins are left behind in the extract solution as they do not bind to the proteins in the hide powder. The non-tannins are determined gravimetrically from the dry mass of the tannin-free extract solution.
- The tannin content is calculated as follows:

Tannin % = Extractives % – (Non-Tannin % + Insoluble %).

- The constituents are expressed as a percentage of the moisture-free or, in this case, freeze-dried bark.
- The red and yellow colors of the bark extract solution are determined within 30 minutes of extraction on a manual Model E Lovibond Tintometer (Lovibond, England). The corrected Lovibond color value is determined by the following equation:

Corrected Lovibond Color of bark extract = (Lovibond color x aliquot of solution filtered x extractives % ÷ tannin % ÷ volume of extraction solution ÷ mass of dry bark).

2.3.6 The effect of pH on the color of the extract solutions

The correlation between pH levels and color is recognized as a factor that impacts the degradation process in tannin extract products (Gulzar *et al.,* 2015). Samples were extracted using deionised water (pH ± 7). Samples from the same batch were subjected to extraction using a pH 4.5 buffered solution of ammonium acetate. The Autoclave method of extraction (Method A4) was used, and the color of the extract solutions was measured**.** The Lovibond color reported is corrected to the amount of tannin present in the milled freeze-dried bark.

The pH of all bark extract solutions was measured using an Orion Star A211 pH meter (Thermo Scientific – United States).

2.3.7 Color and turbidity of the bark extract solutions

Bark extract solutions at a low solid concentration (2-3 %) are unstable due to oxidation that occurs rapidly, resulting in the darkening of the extract solutions (Havemann, 1992). To quantify the rate of darkening of the bark extract solution, the color and turbidity were analysed over time. The samples were stored in a fridge (± 4 ˚C). At specified time intervals, as per Table 2.8 below, an aliquot of the sample was removed and allowed to stabilise to room temperature (± 20 ˚C) before analysis.

Table 2.8 The specified time intervals after which extraction samples were analysed for color and turbidity.

Sample number	Time
1	0 hr (immediately after extraction)
2	0.5 hr
3	1 hr
4	2 hr
5	4 hr
6	6 hr
7	18 hr
8	24 hr
9	168 hr

The bark extract solutions were measured on a Lovibond Model E Tintometer to determine the red color. The color was corrected according to tannin content and reported as red Lovibond units. The relationship between color and time was correlated using polynomial regression in Excel. A 201 Infrared Photometer Lovibond turbidity meter (HACH, South Africa) was used to determine the turbidity calibrated within the range of 20 to 1000 Nephelometric Turbidity Units (NTUs).

2.3.8 The use of additives in the extraction process

Additives are often used in the manufacture of certain wattle bark extract powders. EDTA and oxalic acid ($C_2H_2O_4$) solutions were tested at low (0.1 %) and high (1.0 %) concentrations. This was done to determine whether the use of chelating and reducing agents would enhance the extraction process and inhibit red color formation. Deionised water was used as the control. Fresh bark, i.e., bark that was newly harvested, and aged bark, i.e., bark that was harvested a few days previously and had already begun to oxidise were freeze-dried or oven-dried to create four contrasting samples. The samples were milled, extracted, and thereafter analysed for color using a Lovibond Tintometer to compare statistically.

2.4 Results

2.4.1 Moisture and color determination of freeze-dried and oven-dried bark

The images of oven-dried versus freeze-dried bark in Figure 2.4 demonstrate that freeze-drying (B) inhibited to a large extent the darkening of the bark. The oven-dried (C) bark was much darker than fresh bark (A) or freeze-dried bark (B).

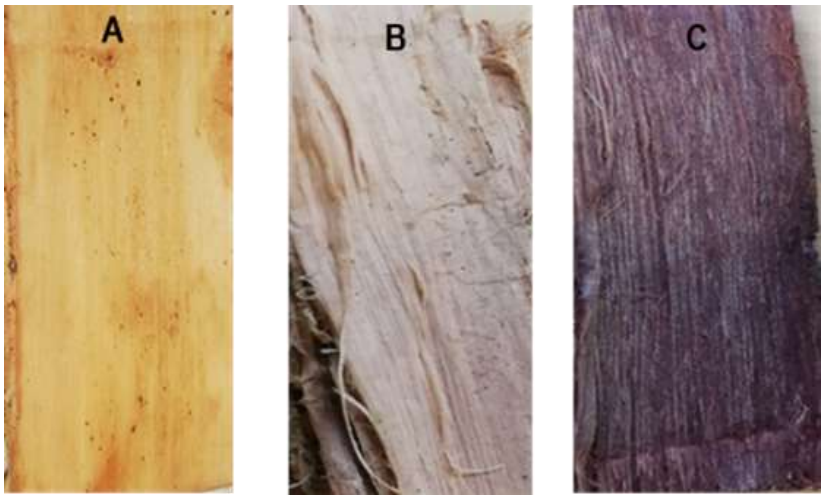


Figure 2.4 The visual comparison of (A) fresh, (B) freeze-dried, and (C) oven-dried black wattle bark.

A paired t-test was used to compare the difference in moisture removal efficacy between freeze-drying and oven-drying of bark before extraction. Results shown in Table 2.9 indicate that the freeze-dying method removed slightly more moisture from the bark than oven drying, although the difference between the methods was not statistically significant ($p = 0.06$). However, freeze-drying resulted in less variability between replicates of the same sample and preserved the color of the bark samples. Hence, freeze-drying was chosen as the optimal method of bark drying.

Table 2.9 A paired t-test for the efficiency of mean moisture removal from black wattle bark by oven-drying versus freeze-drying.

	Oven-drying moisture (%) removal	**Freeze-moisture (%) removal**
Mean moisture %	41.96	43.72
Variance	7.25	5.10
p (T<=t) one-tail	0.06	

2.4.2 Analysis of wattle bark extract solutions, after extraction

The freeze-dried milled bark was extracted using different methods, various chemicals, solvents, and methods of filtration, as described in Methods One to Four, and Experiments One to Nine. The figures and tables in this section show the data obtained from the experiments. Table 2.10 to Table 2.16 shows the mean results for tannin percentage, non-tannin percentage, extractive percentage, tannin/non-tannin (T/NT) ratio, and corrected Lovibond Red color and the p-value for each set of tannin results that was compared to the control results.

S1 compared to the control showed a significant reduction is color with minimal loss of tannins, therefore the control was not carried further as it did not produce the desired outcome. Experiment One (Table 2.10) showed that reducing the extraction time to one hour resulted in the optimal balance between tannin extraction and tannin color. However, any further reduction in time resulted in a significant reduction in extraction efficiency. Therefore, Methods S2 and S3 were not investigated further.

Table 2.10 Analytical and comparative data for Experiment One, evaluating extraction time using the Soxhlet method, presented in comparison to the Control extraction method.

Method	Extractives	Tannin	Non-Tannin	Tannin / Non – Tannin Ratio	[c] Lovibond Red	p-value for Tannin
	%	%	%			
Control	47.3	35.7	11.1	3.2	7.3	
S1 (60 min)	43.6	31.9	11.4	2.8	2.5	0.06
S2 (30 min)	35.2	20.1	14.1	1.4	0.9	< 0.001
S3 (15 min)	27.2	15.6	10.4	1.5	2.0	< 0.001

[c] Low Lovibond Red values are desirable because they indicate that the tannins are pale-colored, which command a premium price.

Experiment Two (Table 2.11) determined the optimal method of filtration after each extraction. Centrifugation (Method S5) compared well to both the control and Method S1 in terms of tannin content, and the color was lighter compared to the control. The filtration method was not carried forward due to the low tannin yield and dark Lovibond color.

Table 2.11 The analytical and comparative data for Experiment Two, comparing filtration methods.

Method	Extractives %	Tannin %	Non-Tannin %	Tannin / Non-Tannin Ratio	Lovibond Red	p-value for Tannin
Control	47.3	35.7	11.1	3.2	7.3	
S1 (Whatman No. 91)	43.6	31.9	11.4	2.8	2.5	0.06
S4 (Whatman GF/C)	33.8	24.9	10.0	2.5	10.1	0.004
S5 (Centrifuge)	44.5	34.40	10.1	3.4	1.3	0.07

Experiment Three investigated the effect of degassing the extract solution and the effect it had on the color of the extract solution. The degassing of the solution in Method S7 by removing the oxygen and replacing it with argon worked as postulated, and the color of the extract solution was lighter. The tannin content was not significantly different from the control, even though it was roughly 10 % lower. The results for Experiment Four are displayed in Table 2.12.

Table 2.12 The analytical and comparative data for Experiment Three testing a modification of the Soxhlet method (control) using Amber glassware or Argon degassed water.

Method	Extractives %	Tannin %	Non-Tannin %	Tannin / Non-Tannin Ratio	Lovibond Red	p-value for Tannin
Control	47.3	35.7	11.1	3.2	7.3	
S6 (Amber glass)	41.6	30.5	11.3	2.7	1.9	0.01
S7 (Degassed)	37.5	27.9	9.6	2.9	1.3	0.09

Experiment Four involved compared the SLTC of extract solutions of the mechanical shaker, as set out in Table 2.4 under the Materials and Methods section. The mechanical shaker allowed for multiple samples to be extracted at once, under gentle conditions. However, the mechanical shaker method was inefficient in extracting tannins. The p-values for tannins were significantly less compared to the Soxhlet control method. Whilst the color of the extracts was lighter than the control, due to their poor extraction efficiency and slowness, these mechanical shaking methods were not tested further. Results are expressed in Table 2.13

Table 2.13 The analytical and comparative data for Experiment Four i.e., the analytical data from the Mechanical Shaker Extracts.

Method	Extractives %	Tannin %	Non-Tannin %	Tannin / Non-Tannin Ratio	Lovibond Red	p-value for Tannin
Control	47.3	35.7	11.1	3.2	7.3	
M1	30,9	23.0	9.2	2.5	4.0	< 0.001
M2	24.0	14.4	8.1	1.8	4.6	< 0.001
M3	25.9	13.7	10.8	1.3	3.1	< 0.001
M4	18.1	9.8	6.5	1.5	1.9	< 0.001

Experiment Five investigated running multiple samples concurrently, aiming to overcome a limitation, which is that the control method is slow, extracting one sample at a time. Method P1 also generated a very light-colored extract, although the tannin content was roughly 10 % less than the control, as shown in Table 2.14.

Table 2.14 The analytical and comparative data for Experiment Five, testing the Pressure cooker treatment for multiple samples.

Method	Extractives %	Tannin %	Non-Tannin %	Tannin / Non-Tannin Ratio	Lovibond Red	p-value for Tannin
Control	47.3	35.7	11.1	3.2	7.3	
P1 (Pressure Cooker + Centrifuge)	48.0	24.7	12.7	2.0	1.0	0.01

Experiment Six investigated the use of a laboratory autoclave. The Autoclave methods produced extraction solutions that had better extraction content than the control and which were paler in color. The results are shown in Table 2.15.

Table 2.15 The analytical and comparative data for Experiment Six, comparing three Autoclave methods at a fixed temperature and pressure for multiple samples.

Method	Extractives %	Tannin %	Non-Tannin %	Tannin / Non-Tannin Ratio	Lovibond Red	p-value for Tannin
Control	47.3	35.7	11.1	3.2	7.3	
A1	55.1	36.2	18.1	2.0	1.4	0.40
A2	51.3	37.6	13.2	2.8	1.2	0.14
A3	57.9	36.5	18.5	2.0	1.1	0.08

Experiment Seven showed that two autoclave extractions were sufficient to remove all the tannins from the bark, which agrees with the literature that suggested two, 15-minute extractions were sufficient (Yazaki *et al.,* 1990). A third extraction made an

insignificant contribution to the final tannin content of the bark extract solution. Figure 2.5 illustrates the absorbance of each extraction using a line graph in Excel.

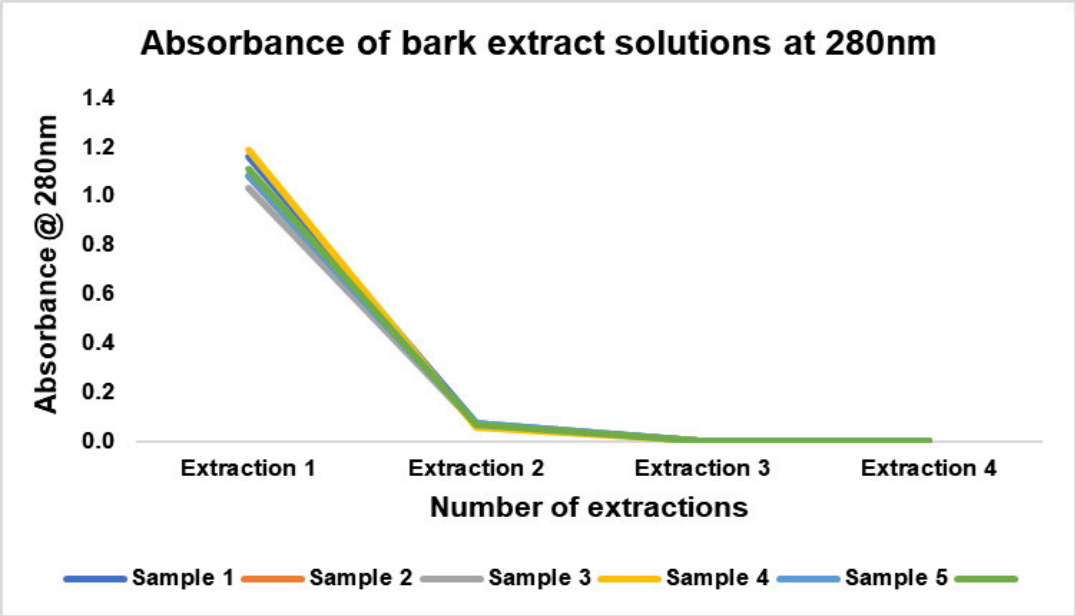


Figure 2.5 The absorbance of multiple extractions (on the same bark) at 280 nm to determine the tannin content in each extraction i.e., Experiment Seven

Given that two extractions were found to be sufficient, Experiment Eight compared Method A3 where three extractions are done to Method A4 where only two extractions are done. Only two extractions were necessary and showed no significant difference from the control in tannin content. The color was also lighter, as shown in Table 2.16.

Table 2.16 The analytical and comparative data for Experiment Eight, comparing Autoclave methods A3 and A4.

Method	Extractives %	Tannin %	Non-Tannin %	Tannin / Non-Tannin Ratio	Lovibond Red	p-value for Tannin
Control	47.3	35.7	11.1	3.2	7.3	
A4 (3 Extractions)	57.9	36.5	18.5	2.0	1.1	0.08
A4 (2 Extractions)	55.4	36.2	17.1	2.1	1.0	0.18

Method S1, S5, S7, A1, A2, A3, and A4 were compared to the control using R (Package: agricolae). These methods are all compared to the control with p-values greater than 0.05. A Fisher's Least Significant Difference (LSD) was also done and the results for tannin percentage and Lovibond Red color are represented graphically in Figure 2.6 (A) and (B), respectively. Methods marked with the same letters do not show significant differences. In Figure 2.6 (A), only Method S7 significantly differed from the other treatments. The remaining methods exhibited comparable tannin percentages, suggesting similar extraction efficiencies. In Figure 2.6 (B) the color of the control was by far the highest, significantly worse than all other treatments. Method A4 had the lowest value, significantly different from Methods A1, S1 and S5. However, its LSD value overlapped with Methods S7, A2 and A3.

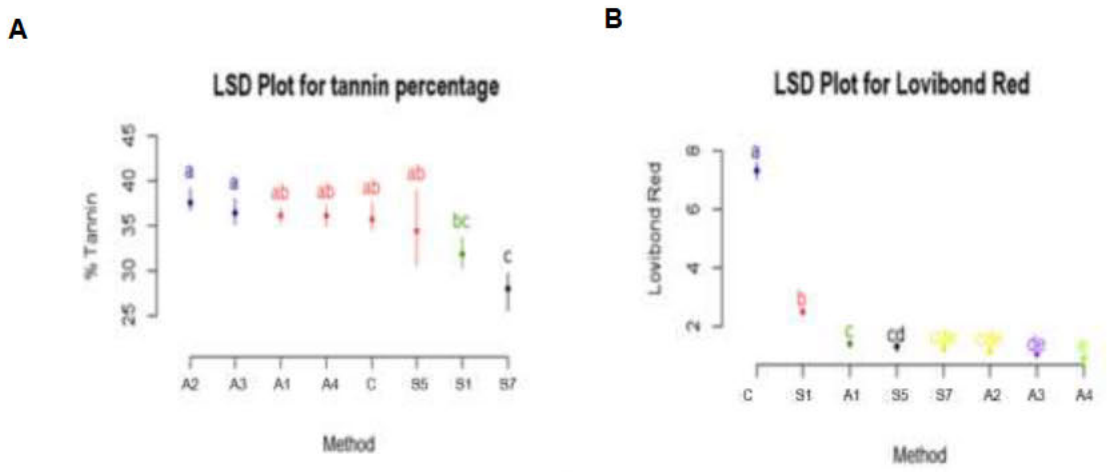


Figure 2.6 LSD plot for tannin percentage (A) and Lovibond Red (B). Different letters indicate significant differences among methods based on Fisher's LSD test at $\alpha = 0.05$. Sharing at least one letter are not significantly different, and treatments that show exactly the same letter (e.g., A2 and A3 for LSD tannin percentage both have an "a") shows no difference.

A two–factor ANOVA (the data was generated from a balanced experimental design) was done in Excel, and the variance for each quality parameter of each method is listed in Table 2.17. The Autoclave methods A3-A4 showed low levels of variance for

all constituents in comparison to the control. Method A3 was the best method in terms of variance for tannin percentage, however, when compared to Method A4, the difference in variance was insignificant. The disadvantage with Method A3 is that it requires three extractions of 30 min each, taking 90 minutes total. In contrast, Method A4 uses three extractions of 15 min, taking 45 min to be completed. Method A4 can be used to process twice as many samples as Method A3 at the same time with a non-significant loss of tannin content and a better color quality. Therefore, Method A4 was chosen because it is a rapid method that does not compromise on the tannin content or color of the bark extract solution.

Table 2.17 The variance was determined by a two-factor ANOVA for quality parameters of bark extract solution determined by the SLTC method of analysis.

Method	**Extractives**	**Tannin**	**Non - Tannin**	**Insoluble**	**Tannin/ Non-Tannin Ratio**	**Lovibond Red**	**Lovibond Yellow**
	%	**%**	**%**	**%**			
Control	2.20	1.72	0.72	0.59	0.04	0.06	1.85
S1	2.78	3.40	0.20	0.03	0.06	0.01	0.03
S5	5.05	12.42	5.05	0.13	1.67	0.02	0.03
S7	1.93	4.38	0.68	2.26	0.19	0.01	0.02
A1	12.30	12.07	1.80	0.70	0.01	0.02	0.17
A2	0.05	0.71	0.16	0.21	0.01	0.01	0.17
A3	0.41	1.55	0.35	0.20	0.02	0.01	0.01
A4	1.87	1.04	0.22	0.56	0.01	0.01	0.01

Experiment Nine examined the sample filtration options. Method A4 had a relatively high insoluble content (greater than 2 %) because of its use of centrifugation, allowing larger particles to remain in the filtrate. A t-test was done to compare the tannin and color using Method A4, compared with a modified version of the method where samples were passed through a Buchner funnel filtration setup. However, there was no significant difference in tannin content between the two methods ($p = 0.05$). The results are displayed in Tables2.18 and 2.19 respectively.

Table 2.18 The paired t-test analysis for tannin % of Method A4 to compare filtration of the extract solution using centrifugation and a Buchner funnel.

	Centrifugation of extract solution	**Filtration of extract solution using a Buchner funnel**
Mean tannin %	35.73	36.18
Variance %	0.40	0.37
p(T<=t) one-tail	0.05	
t Critical one-tail	1.83	

Table 2.19 displays the t-test results for the Lovibond Red color comparing the standard Method A4 centrifugation with subsequent filtration of the extract solutions. There was a significant difference in the color of the extracts between the two methods. The A4 method using centrifugation only has a lower Lovibond color. Given that the color of the extract is highly significant in the extraction process, it was established that centrifugation alone worked best.

Table 2.19 The paired t-test analysis for Lovibond color (red) of Method A4 to compare filtration of the extract solution using centrifugation and a Buchner funnel.

	Centrifugation of extract solution	Filtration of extract solution using a Buchner funnel
Mean Lovibond red	2.28	2.77
Variance %	0.01	0.01
p(T<=t) one-tail	1.31×10^{-7}	
t Critical one-tail	1.83	

2.4.3 The effect of pH on the color of the extraction solution

The effect of the extraction solution pH was also investigated to determine if a pH-buffered solution would enhance the color. Figure 2.7 is a bar graph that shows the effect that pH has on the color of the solution. It was noted that deionised water worked best, and that the buffered solution degraded the color of extracts.

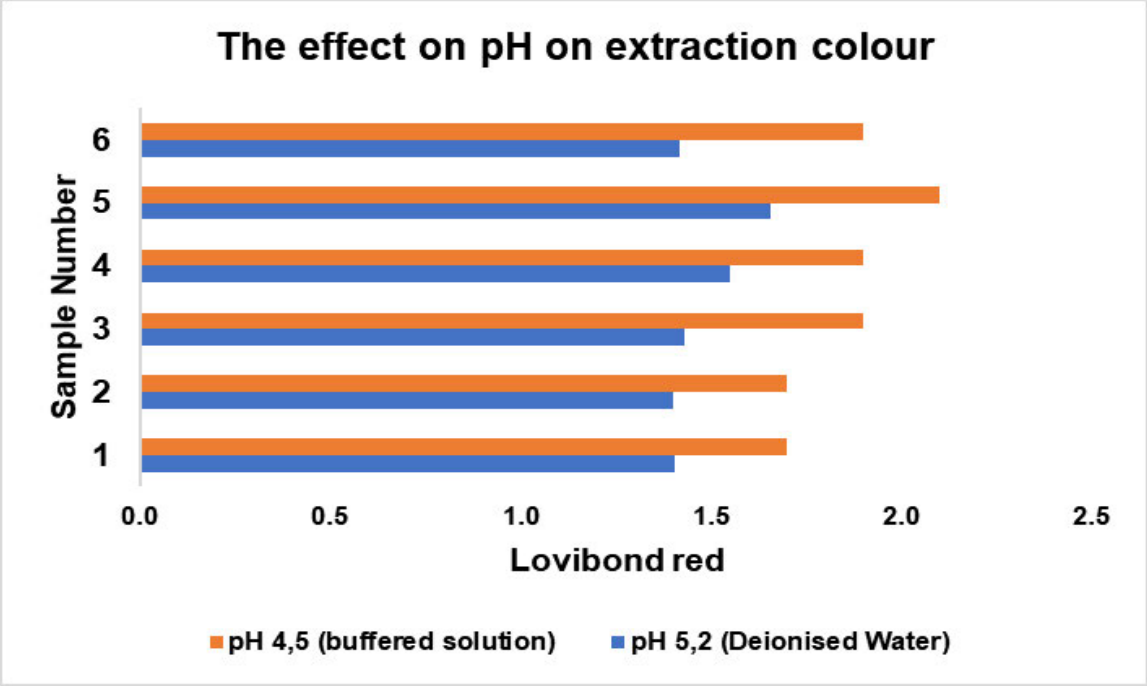


Figure 2.7 The effect of pH on bark extraction solution color using deionised water and an ammonium acetate buffer solution.

2.4.4 Color development and turbidity over time of the extract solution

The milled freeze-dried bark samples underwent extraction using the Autoclave method (Method A4). In Figure 2.8, an exponential trendline was applied. Over time, color development was observed as particles began to precipitate. Consequently, it was concluded that extracts should be analysed within 30 minutes post-extraction, as illustrated in the subsequent figures.

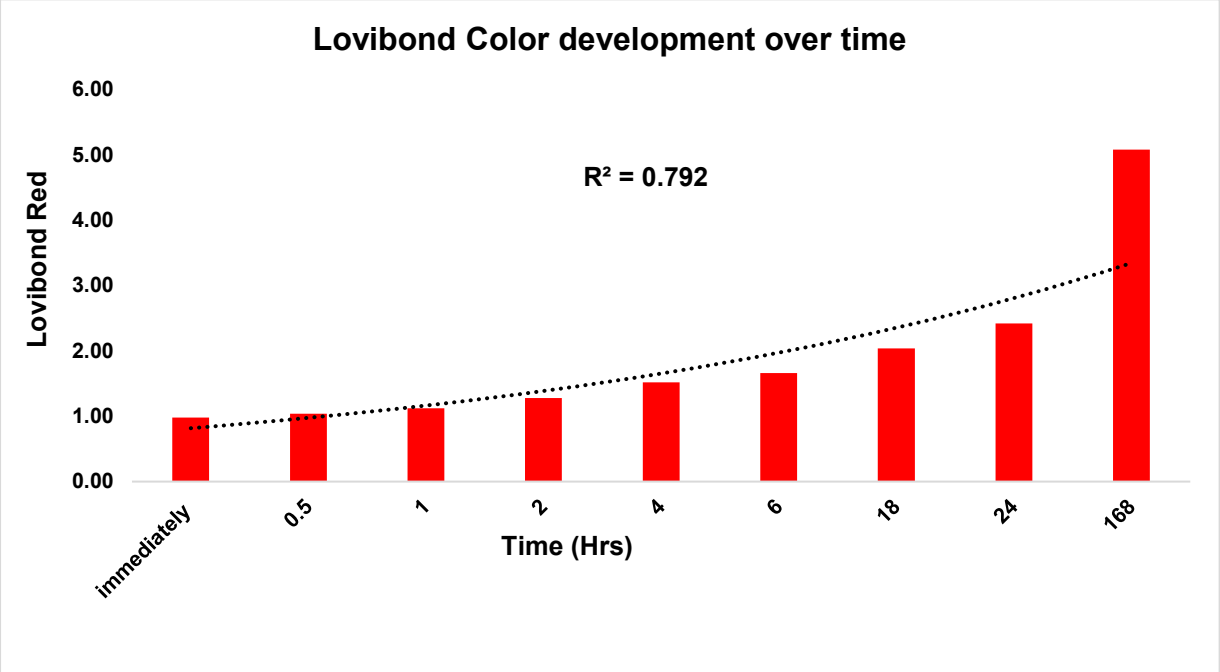


Figure 2.8 A bar graph to illustrate the Lovibond Red color determination over time (The color was corrected using the tannin content of the extraction solution). The dotted line is an exponential fitted trendline showing a significant increase in color over time.

In Figure 2.9, a linear regression trendline was employed to demonstrate the increase in turbidity over time. The data showed that turbidity increased as the duration increased, with a larger standard deviation observed in samples that were allowed to stand for extended periods.

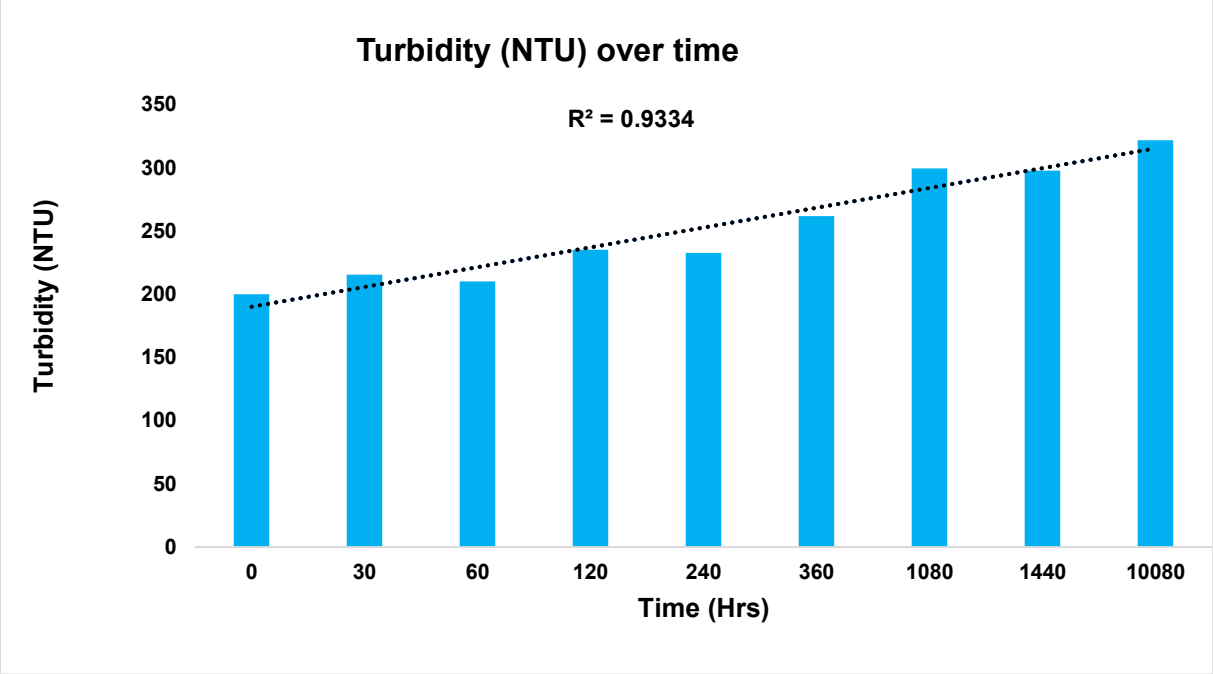


Figure 2.9 A bar graph to illustrate the turbidity (NTU) determination over time (The turbidity of the extraction solution is reported on an as-is basis). The dotted line represents a linear regression trendline, showing that turbidity increases with time.

2.4.5 The use of additives in the bark extraction process

The use of freeze-drying combined with various additives produced diverse outcomes. Specifically, adding 0.1 % EDTA to the bark extract influenced the color of the solutions visually, yielding lighter-colored extracts as shown in Figure 2.10.

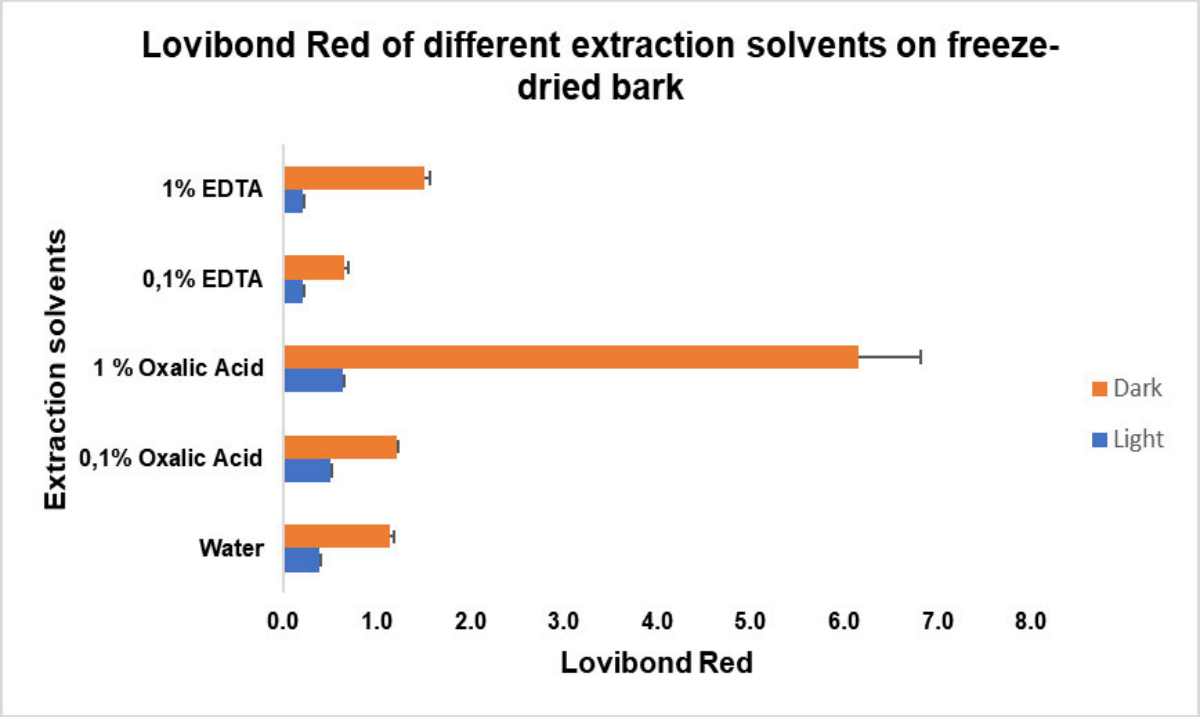


Figure 2.10 Lovibond Red color of freeze-dried bark solutions using additives in the extraction process of light bark (fresh) and dark bark (aged). The bars represent the standard deviation.

In Figure 2.11, the oven-dried bark exhibited a darker color. The inclusion of various additives led to differing results, with the aged bark showing a higher standard deviation in color darkness. Notably, the addition of 0.1 % EDTA consistently resulted in the lightest colored extract.

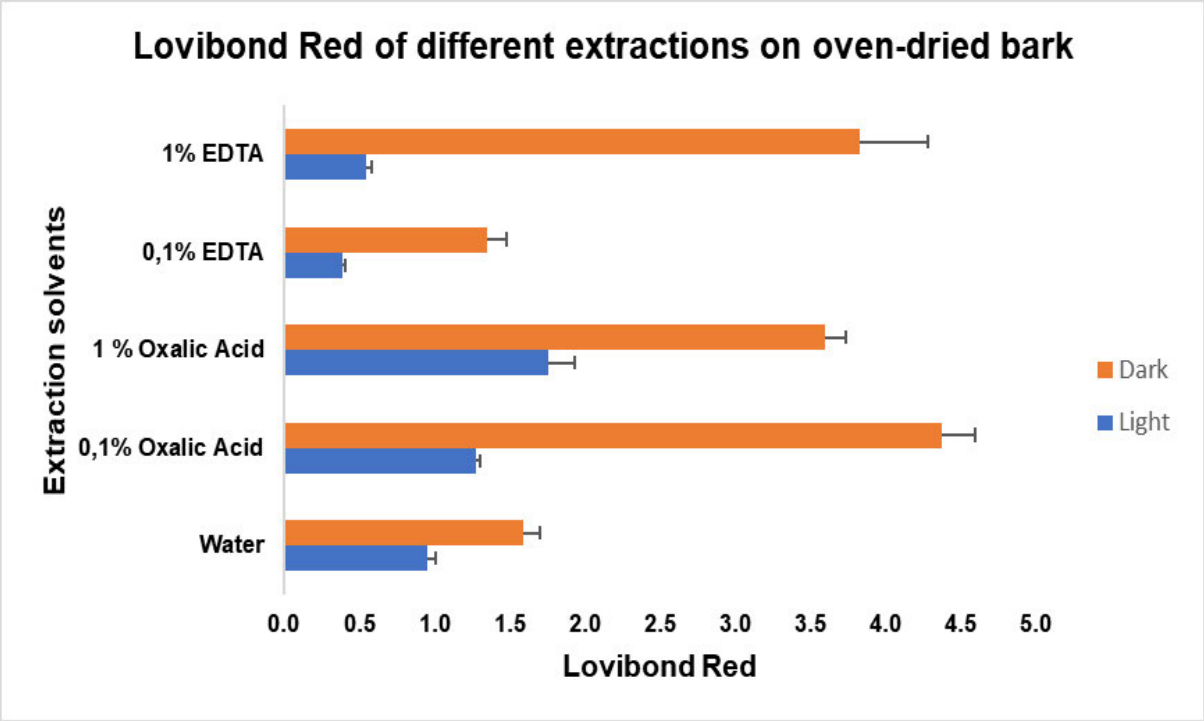


Figure 2.11 Lovibond Red color of oven-dried bark solutions using additives in the extraction process of light bark (fresh) and dark bark (aged).

2.5 Discussion

The existing standard laboratory extraction process for wattle bark primarily aims to maximise the tannin content in the extraction liquid. Yet, other important factors include speed, extract color, extractives (compounds removed during the extraction process, inclusive of the tannin and non-tannin fractions), insoluble, and non-tannins. The primary objectives in developing a new, high-throughput method for laboratory analysis of black wattle bark characteristics were to expedite the process while maintaining the lightest extract color and ensuring that extraction efficiencies remain on par with current laboratory and industrial practices.

Moisture removal by freeze-drying proved to be an important step in this process. Freeze drying was slightly more efficient (by preventing color degradation, moisture determination showed no significant difference(Figure 2.4 and Table 2.9)) than the conventional method of oven-drying bark used by the factories and the freeze-dried bark did not darken (Figure 2.4), because freeze drying arrests color changes (Jain *et al.,* 2016) after bark harvesting. In addition, since extractable tannin content in plant material decreases when exposed to high temperatures (temperatures of 60˚C and above), it is recommended that oven drying of tannin material should be avoided

(Gupta *et al.,* 2012). This also suggests that oven-drying methods can introduce errors in wattle bark laboratories determination of bark tannin and color content. This will also imply that a major change in bark processing in wattle bark factories would be needed, with their consideration for installing freeze-drying units.

After determining the optimal drying method to store material without degradation, a sequence of elimination experiments was used to determine the best method of extraction. This was designed with the intention of improving on the current method by evaluating each step of the method. The current method is a harsh process due to the continuous boiling of the sample (Silva *et al.,* 1998); therefore, results can vary. It is also a slow process as only one sample can be extracted at a time. The ideal outcome of this research was to discover a method of extraction that would allow for multiple samples to be extracted concurrently with less variation, little or no additional cost, and with no loss in efficiency.

The first experiment was to investigate if reducing the time of the Soxhlet extraction affected the tannin content and color. The control method is tedious, with a low sample throughput and excessively dark colors. The experiment was successful as it reduced the time to one hour per extraction. Because any further reduction in time produced inconsistent yields and low tannin content, the one-hour period for a single extraction was determined to be optimal. This can greatly reduce current extraction times and produce results consistent with existing methods, without the excessive darkening of the solution, so that sources of a darker color can be traced back to problems at the source of the bark, without being masked by the extraction process. This could help improve bark quality monitoring and extract quality at the factory.

The use of filter paper in the Soxhlet extraction process was investigated because the composition of the filtering material can affect tannin yield, i.e., cellulose can immobilize tannin (Li *et al.,* 2022). Interestingly, the GF/C filter paper, which is made of glass fibres, increased undesirable color development. Overall, the color of the extracts was darker, and the insoluble content were much lower than the other experiments, as expected. This is due to the interaction of the filter paper and tannins, whereby the tannins bind to the cellulose in the filter paper (Oh *et al.,* 1980). The surface area of the filter paper also allows for more oxidation to occur (Mohan and Karthikeyan, 1997), producing a darker tannin red color. Oxidation also

decreases the permeability of the tannins, resulting in an even darker solution because the time for filtration is increased (Halloin, 1982). Therefore, it was found that filtration with filter paper was not suitable for an optimized extraction and a different method of removing particulate matter from the extract solution was required. Centrifugation was found to efficiently remove larger particles, which settled at the bottom of the centrifuge tube. The centrifuge method was also quicker than the filtration method. Therefore, it was determined that sample centrifugation after each extraction was more efficient than filtration and did not contribute to color degradation.

The existing Soxhlet methods were evaluated for potential improvements to justify their use. Removing oxygen from the extraction solution using Argon gas during extraction showed some improvement in color quality compared to the control. However, the method is highly expensive, requiring hundreds of Argon canisters per season, making it cost-prohibitive. Additionally, the process is slower and more cumbersome, offering minimal overall benefit.

The use of amber glassware to prevent light-induced tannin oxidation during extraction was investigated. However, the method produced inconsistent results due to the material adhering to the walls of the reaction vessels and often burning, resulting in varied results. Powdered material (milled freeze–dried bark), essential for these experiments for testing homogenized bark samples, was not suitable for Soxhlet extraction. Typically bark chips used in a factory laboratory, do not adhere to the glass. Therefore, these results suggest that these methods to try inhibiting the darkening of the extract solution were impractical and did not significantly benefit color preservation during Soxhlet extractions.

Alternative mechanical extraction approaches to heating were explored because heat is a known cause of tannin darkening (Roux *et al.,* 1975). A mechanical shaker was used as a gentle extraction method with cold water. However, the mechanical extraction had a very low extraction efficiency, took a much longer time (16 h), and a single extraction was not sufficient to extract sufficient levels of tannin. While cold water extractions have been used elsewhere, it has been found that condensed tannins are not adequately extracted (Ding *et al.,* 2017). Hot water was also tested using the mechanical extraction process; however, it produced low extractives and

high color, which is undesirable. This method did not achieve the desired goals of the novel method developed in this work.

It has been found that although water is a good solvent for the extraction of tannins, the mixture of water and organic solvents can produce better extraction efficiencies (Maitera *et al.,* 2018)**.** Therefore, water and ethanol mixtures were tested as extraction solvents to determine whether they would enhance the extraction, because ethanol as an extraction solvent has been proven to improve the tannin yield (Abilleira *et al.,*2021). Although, tannin extraction was enhanced relative to water only, the tannin yield was significantly lower than the control. Ethanol and methanol are used for tannin extraction in labs, whereby the extract solutions are further analysed using High-Pressure Liquid Chromatography (HPLC) or Gas - Chromatography (GS). However, these methods are impractical for bulk samples, are time-consuming, expensive, and only analyse for tannin chemical composition.

The Pressure Cooker method P1 produced unsatisfactory results as the tannin content did not correlate to the control, it was 10 % lower, although the color value was low. Therefore, this method was not investigated further. The temperature and pressure varied because the electronically controlled, domestic pressure cooker is not a scientific instrument, and it lacks precision. However, it did point towards the fact that high-pressure extraction, as is used in factories, is a good option, justifying the investigation of laboratory autoclaves.

The use of an autoclave was evaluated in terms of time and number of extractions. The wattle extract factories use similar technology for bark extractions on a larger scale, whereby the fresh bark chips are extracted in large autoclaves at fixed temperatures and pressure. Multiple extractions from the same batch of bark are done to remove as much tannin as possible (Havemann, 1992).

All the Autoclave methods outperformed the Soxhlet control in extraction efficiency (% extractives), tannin content, and in color quality. The color of the extraction using the average of all four autoclave methods compared to the traditional Soxhlet showed a 70 % reduction in color degradation. When the average Autoclave method was compared to the modified Soxhlet (Method S5 - shorter time and samples centrifuged instead of filtered), there was a 10 % reduction in color degradation.

Experiments A1, A2, A3, and A4 (Autoclave Methods) all showed similar extraction efficiency, tannin %, and low Lovibond Red color. Autoclave experiment A4 was the best method overall because it provided a compromise between speed, practicality, and adequate extraction, with the tannin content being comparable with the control, and the extract retained a pale color (low Lovibond Red, i.e., better color quality). The method also allowed for multiple samples to be extracted at once (12 – 20 samples at once). The Method A4 will be referred to as the Double Autoclave Water (DAW) Method in future experiments.

The insoluble percentage for the DAW method was on average 2 %, which was higher than the control method. However, when assessing methods of filtration, it was determined that the tannin percentage was not affected, and the extract color was best without filtration. Therefore, the important measured quality parameters were not affected. The T/NT (tannin/non-tannin ratio) was also within range, with a value of 2.4, which indicates an adequate level for bark and extract powder quality (Chan *et al*.,2015).

An experiment on the effect of pH on tannin extract solutions showed that buffered acidic solutions resulted in darker-colored extracts compared with deionised water. Buffered acidic solutions also had a high turbidity, due to an excess of particles in the solution, which affects color analysis (Bennett and Drikas, 1993). Alkaline solutions were not considered as they are known to produce low extraction efficiency and darker color (Churms and Stephen, 1991). Hence, deionised water was used in subsequent extractions.

Tannin content and color are affected by chemical changes over time (Gong *et al.,* 2014). Oxidation and fermentation that occur over time will result in increased color development, i.e., darkening (Schofield *et al.,* 2001). Therefore, the samples ideally need to be analysed within 30 mins of the extraction to ensure consistent color analysis as any further delays lead to the precipitation of the tannins out of solution, higher turbidity, and color development, as illustrated in Figures 2.8 and 2.9. This is a strong argument for the development of a rapid low-cost method such as near infrared reflectance spectroscopy, which can be used to determine tannin concentrations (Yazaki *et al.,* 1993) to avoid the need for lengthy and laborious SLTC analysis and the associated technical errors that could occur.

In addition to the physical and chemical characteristics of a natural bark solution, the addition of chemical additives could mitigate the oxidation reaction responsible for darkening to preserve ideal color properties. Figures 2.10 and 2.11 showed that the addition of 0.1 % EDTA had a positive effect on bark extraction, in comparison to water and oxalic acid. The addition of EDTA enhanced the color of the bark extract. EDTA and oxalic acid are both reducing agents, so it was surprising to note that oxalic acid performed negatively. EDTA is a chelating agent that binds to iron, which results in a lighter extract because iron forms an iron-tannate complex in solution, allowing hydroxyl ions to attach to the complex, causing oxidation and darker colors (South and Miller,1998). When the EDTA binds the iron, it prevents further reactions, which would prevent further color darkening caused by iron-tannate complexes (Baynes and Bothwell, 1990). The lightening effect of EDTA in the extraction process is a key finding and is worth exploring on an industrial scale, together with the testing of other chelating agents.

This study demonstrated that the use of freeze-drying was critical to arrest color development in wattle bark so that color analysis properties of bark could be determined. The DAW method was identified as a faster and more efficient extraction method that can be used to replace the traditional reflux method for the assessment of wattle bark quality parameters. Also, the use of EDTA was identified as a beneficial step that could enhance tannin extract quality. Using these improvements to the bark tannin analysis, future research could develop methods, such as near-infrared spectroscopy (NIRS), to evaluate the quantity and quality of tannins in fresh chipped bark, before drying.

2.6 References

Abilleira, F., Varela, P., Cancela, Á., Álvarez, X., Sánchez, Á. and Valero, E. Tannins extraction from *Pinus pinaster* and *Acacia dealbata* bark with applications in the industry. *Industrial Crops and Products*, vol. 164, pp. 113-394, 2021.

Bae, H.D., McAllister, T.A., Yanke, J., Cheng, K.J. and Muir, A.D. Effects of condensed tannins on endoglucanase activity and filter paper digestion by *Fibrobacter succinogenes* S85. *Applied and Environmental Microbiology*, vol.*59*(7), pp. 2132-2138, 1993.

Baynes, R.D., and Bothwell, T.H. Iron deficiency. *Annual Review of Nutrition* vol. 10 (1), pp. 133-148, 1990.

Bennett, L.E. and Drikas, M. The evaluation of color in natural waters. *Water Research*, vol. *27*(7), pp. 1209-1218, 1993

Bhagwandin, A. Personal communication with Mr. A. Bhagwandin, Production Chemist, NTE Hermannsburg, Hermannsburg, January 2022.

Chan, J.M., Day, P., Feely, J., Thompson, R., Little, K.M. and Norris, C.H. *Acacia mearnsii* industry overview: current status, key research, and development issues. *Southern Forests: A Journal of Forest Science*, *77*(1), 19-30. 2015

Chaunbi, G. Black wattle plantations in South Africa: Introduction, Silviculture, and Management. In: *Black Wattle and its Utilisation*. Research Institute of Subtropical Forestry, Chinese Academy of Forestry Fuyang Zhejiang, China. Pp 33 -39, 1997.

Churms, S.C. and Stephen, A.M. Chromatographic separation and examination of carbohydrate and phenolic components of the non-tannin fraction of black wattle (*Acacia mearnsii*) bark extract. *Journal of Chromatography A*, vol. *550*, pp. 519-537, 1991.

Ding, T., Bianchi, S., Ganne-Chédeville, C., Kilpeläinen, P., Haapala, A. and Räty, T. Life cycle assessment of tannin extraction from spruce bark. *iForest: Biogeosciences and Forestry*, vol. *10*(5), pp. 807-814, 2017.

Dunlop, R.W. and MacLennan, L.A. Chapter 1: Introduction, Black *Wattle: The South African Research Experience*. Institute for Commercial Forestry Research, Pietermaritzburg, South Africa. Pp 1-5, 2002.

Gong, X., Li, Y. and Qu, H. Removing tannins from medicinal plant extracts using an alkaline ethanol precipitation process: a case study of Danshen injection. *Molecules*, vol. *19*(11), pp. 18705-18720, 2014.

Gujrathi, A.M. and Babu, B.V. Environment-friendly products from black wattle. *Energy Education Science and Technology*, vol. *19*(1), pp. 37-44, 2007.

Gulzar, T., Adeel, S., Hanif, I., Rehman, F., Hanif, R., Zuber, M. and Akhtar, N. Eco-friendly dyeing of gamma ray induced cotton using natural quercetin extracted from acacia bark (*A. nilotica*). *Journal of Natural Fibers*, vol. *12*(5), pp. 494-504, 2015.

Gupta, A., Naraniwal, M. and Kothari, V. Modern extraction methods for preparation of bioactive plant extracts. *International journal of applied and natural sciences*, *1*(1), pp.8-26, 2012.

Halloin, J.M. Localization and changes in catechin and tannins during the development and ripening of cottonseed. *New Phytologist*, vol. *90*(4), pp .651-657, 1982.

Havemann, D.A. *The Manufacture and Uses of Wattle Bark Extract*. Ph.D. thesis, University of Stellenbosch. Pp 31-48, 1992.

Hurlow, E.L. *Aspects of the Anaerobic Digestion of Wattle Tannins*. MSc dissertation, Department of Chemistry. Rhodes University. Grahamstown. Pp 21-29, 1986.

Jain, R., Janakiram, T. and Kumawat, G.L. *Drying Techniques in Ornamental - Plants.* Division of Horticultural Science, Indian Agricultural Research Institute, New Delhi, India. Pp 501-512, 2016.

Leafe, M.K. (Ed.). *Leather Technologist's Pocketbook.* Society of Leather Technologists and Chemists, United Kingdom. Pp 227-272, 1999.

Li, A., Liu, J., Qin, Z., Wang, L., Li, L., Tang, K., and Pei, Y. Black wattle tannin-immobilized mesostructured collagen as a promising adsorbent for cationic organic dyes (methylene blue) removal in batch and continuous fixed-bed systems. *Journal of Applied Polymer Science*, vol. *139*(26), p.e52452, 2022.

Oh, H.I., Hoff, J.E., Armstrong, G.S. and Haff, L.A. Hydrophobic interaction in tannin-protein complexes. *Journal of Agricultural and Food Chemistry*, vol. *28*(2), pp. 394-398, 1980.

Maitera, O.N., Louis, H., Oyebanji, O.O. and Anumah, A.O. Investigation of tannin content in Diospyros mespiliformis extract using various extraction solvents. *Journal of Analytical & Pharmaceutical Research*, vol. *7*(1), pp. 55-59, 2018.

Mohan, S.V. and Karthikeyan, J. Removal of lignin and tannin color from aqueous solution by adsorption onto activated charcoal. *Environmental Pollution*, vol. *97*(1-2), pp. 183-187, 1997.

Roux, D.G., Ferreira, D., Hundt, H.K. and Malan, E. Structure, stereochemistry, and reactivity of natural condensed tannins as basis for their extended industrial application. *Journal of Applied Polymer Science,* vol. 28, pp. 335-353, 1975.

Schofield, P., Mbugua, D.M. and Pell, A.N. Analysis of condensed tannins: A Review. *Animal Feed Science and Technology*, vol. *91*(1-2), pp. 21-40, 2001.

Sherry, S.P. *The Black Wattle* (*Acacia mearnsii* de Wild). University of Natal Press, Pietermaritzburg, South Africa. Pp 20-33, 1971.

Silva, G.L., Lee, I.S. and Kinghorn, A.D. Special problems with the extraction of plants. *Natural Products Isolation,* vol. 4, 343-363, 1998.

Slabbert, N. Complexation of condensed tannins with metal ions. In *Plant Polyphenols*. Springer, Boston, MA, USA. Pp 421 – 436, 1992.

South, P.K. and Miller, D.D. Iron binding by tannic acid: effects of selected ligands. *Food Chemistry*, vol *63*(2), pp. 167 – 172, 1998.

Yazaki, Y., Guangcheng, Z. and Searle, S.D. Extractives and polyflavanoid contents of *Acacia mearnsii* bark in Australia. *Australian Forestry*, vol. *53*(3), pp. 148-153, 1990.

Yazaki, Y., Gu, R., Lin, Y., Chen, W., and Nguyen, N. Analyses of black wattle (*Acacia mearnsii*) tannins: relationships among the hide-powder, the Stiasny, and the ultra-violet (UV) methods. *Holzforschung*, vol .*47*(1), pp .57-61, 1993.

Chapter 3: Near-Infrared Spectroscopic (NIRS) analysis of black wattle (*Acacia mearnsii* de Wild.) bark quality parameters

3.1 Abstract

Since the early 1900s, the Society of Leather Technologists and Chemists (SLTC) has used the hide powder method to evaluate tannin content and quality traits in wattle bark and Mimosa products. This approach has become the global standard in both the wattle bark and leather industries. Alternative methods, such as the Stiasny method, are predominantly employed for estimating adhesive tannin content, and ultraviolet (UV) spectroscopy is applied in both adhesive and tanning processes. However, Near-Infrared Reflectance Spectroscopy (NIRS) has emerged as a highly efficient, cost-effective, and non-destructive analytical tool. NIRS is widely used across various industries, including food and agriculture, and has proven its effectiveness for tannin analysis. This study explored the application of NIRS using different sample preparations, such as milled freeze-dried bark and bark extract solutions. By integrating NIRS scans with traditional SLTC methods and elemental analysis, models were developed to predict key properties of wattle bark and its extracts. UV spectroscopy was also tested for tannin determination but showed limited accuracy compared to NIRS. Notably, the NIRS analysis of milled freeze-dried bark demonstrated strong predictive performance for all critical quality parameters. These findings underscore the potential of NIRS as a viable alternative to traditional wattle bark analysis techniques. Its accuracy, speed, and non-destructive nature make it a promising replacement for existing methodologies, offering enhanced efficiency for tannin content and quality analysis in the industry.

3.2 Introduction

Tannins are water-soluble, polyphenolic substances found in a wide variety of vegetation where they play an important role in protecting the plant against free radicals caused by light and biological attacks (Hagiopol, 2021). They are divided into two groups: condensed tannins and hydrolysable tannins. Condensed tannins are used by humans in the manufacture of pharmaceuticals, animal feeds,

adhesives, and specialty chemicals used in various industries such as the leather manufacturing industry (Hemingway and Karchesy, 2012).

Tannin extract from black wattle bark is a significant export from the KwaZulu-Natal and Mpumalanga regions of South Africa, accounting for USD 60 million p.a. in exports, mainly for use in the leather tanning industry (Chan *et al.,* 2015). The characteristics of wattle bark and the extract products are measured for quality assurance and monitoring purposes. The primary quality characteristics of tannin powder extracts, such as Mimosa ME, are the tannin content and colour (Gujrathi and Babu, 2007).

The leather industry currently determines the tannin content of bark extracts using the SLTC method, which is based on the protein binding activity of tannin. An expensive purified form of animal hide powder (BLC Leather Technology, USA) is used to determine the quantity of tannin-binding polyphenolics in a tanning product. The process is laborious, and it can take days to analyse a single sample, making the processing of large numbers of samples impractical (Gordon-Gray, 1953). Furthermore, the method is not consistent, with as much as a 2 % absolute difference in reported tannin levels for different hide powder tests on the same sample (Garbutt and Nobel, 1983).

Several studies have employed spectroscopic methods to estimate the quantification of total phenolics and protein-binding assays for tannin extracts (Hagerman and Butler 1989). The major components of wattle tannins are polyflavonoids, which strongly absorb light in the ultraviolet region of 250—280 nm (Tindale and Roux, 1969). Yazaki *et al.,* (1993) compared the UV, Stiasny, and hide powder methods. They found that the UV spectroscopic method is quick and simple and has a high throughput but tends to overestimate tannin content. The problem is that not all polyphenolics have tanning properties, and other compounds also absorb light at 280 nm, including other phenolic compounds and proteins (Chang and Zhang, 2017). Therefore, the UV method would overestimate the tannin content because it also detects phenolic non-tannins (i.e., mono-flavonoids and di – flavonoids) that do not bind to the hide powder method (Roux, 1957).

The Stiasny method uses a formaldehyde – tannin reaction to analyse tannin content. The Stiasny reaction results in lower estimates of tannin levels although it

can be used for rapid estimations is typically applied for bark extract products that are used to produce adhesives (Ogawa and Yazaki, 2018). The Stiasny method and UV spectroscopy could be used for screening of bark for silvicultural purposes where many samples need to be analysed (Yazaki *et al.,* 1993). However, this approach is not suitable for leather tanning properties.

Other chemistry methods of analysing leather tanning content have been explored. Garbutt and Nobel (1983) tested the use of dimethylaminoethyl cellulose instead of hide powder. This method did not require complex and potentially toxic hide powder preparations, and the binding agent was reusable. However, this form of cellulose does not appear to be commercially available, although variants thereof could be tested in its place (Garbutt and Nobel, 1983). Antoine *et al* (2004) suggested the use of Divergan™ (polyvinylpolypyrrolidone) as a method for tannin content determination. The Divergan™ technique is based on the precipitation of tannins by their reaction with polyvinylpyrrolidone. The method compares well to the hide powder method. However, this is not the wattle industry standard (Antoine *et al.,* 2004). Therefore, tannin content analysis after bark extraction can still pose challenges for commercial tannin extract production because it needs to relate to the SLTC hide-powder method.

A more suitable candidate method for tannin analysis is NIR spectroscopy (NIRS). NIRS can be described as a human and eco-friendly technique. NIRS has many applications such as quantitative analysis, identification, quality control, quality assessment, prediction of physical and chemical properties, and online monitoring (Ozaki, 2022). NIRS offers a low-cost, non-destructive method of analysing pharmaceutical and agricultural products. This technology depends on the quantitative infrared light absorbing properties of organic compounds. Regression models to predict the quantities of organic compounds are developed using conventional multivariate analysis, or machine learning models, and chemometrics (Siesler *et al.,* 2008).

Schimleck and Yazaki (2003) compared the UV, Stiasny, and hide powder methods in conjunction with NIRS. They showed that NIRS could be used to estimate the tannin content directly in black wattle bark. They analysed nine different chemical factors in black wattle bark, including the tannin content. They obtained coefficients

of determination (R^2) values that were greater than 0.8. They concluded from their study that NIRS showed considerable potential for the assessment of quality parameters in wattle bark (Schimleck and Yazaki, 2003). However, they did not analyse or develop a method to determine the colour of wattle bark.

Near-Infrared Transmission Spectroscopy (NITS) was also tested as an SLTC-derived approach to analyse bark extract solutions, testing transmission, and reflectance modes to allow for the rapid and inexpensive determination of multiple quality parameters at once. NIRS models for milled freeze-dried samples were also developed to replace laborious and costly extractions and SLTC analysis, for future research and laboratory quality control. Model performance was evaluated using the coefficient of determination (R^2), the Ratio of Prediction to Deviation (RPD), and the Ratio of Performance to Interquartile Range (RPIQ). According to Menezes et al. (2014) and Xiaobo *et al*. (2010), values exceeding 80% for R^2, greater than 2 for RPD, and greater than 2.5 for RPIQ are indicative of good model performance. These benchmarks provided a reliable basis for investigating the suitability of NIRS as a rapid, non-destructive alternative for bark quality evaluation in both the research and commercial or industrial settings.

3.3 Materials and Methods

3.3.1 Bark sample collection at Harden Heights

Black wattle bark samples were collected from ten-year-old trees at Harden Heights, KwaZulu-Natal (GPS coordinates - 29.2667° S, 30.6167° E). The samples were collected every second month, at the start of the rainy season, from September 2020, until July 2021 when the rainy season ended, and industry harvesting stopped because wattle bark is difficult to strip from the wood when during the dry season. Four trees were sampled at every sampling event in a 24-hour sampling period. Bark samples were taken as a 10 cm high band around the entire stem using a stainless-steel cleaver to reduce the potential for iron contamination, which can affect colour (Slabbert, 1992). The bark samples were placed in plastic bags, vacuum-sealed, and placed in a cooler box with ice blocks to prevent degradation. The samples were transported to the Institute for Commercial Forestry Research (ICFR) laboratory within 2 hours for further processing.

3.3.2 Bark treatment and ageing

The samples were divided into two groups, i.e., fresh bark (t = 0 days) and treated bark. The fresh bark group samples were weighed to record wet mass, and then immediately placed into a Virtis BT Pro series Freeze dryer (Lab 1ST, USA) at -70 ˚C and 175 millitorrs (mTorr) for 48 hours until a constant mass was achieved. The mass after drying was taken to determine the moisture content.

The treated bark underwent light and water exposure, and combinations thereof for two (t = 2 d) and four days (t = 4 d) in two incubators (Labcon, Low-Temperature Incubator, Model LTIE) that were set to 15 ˚C and 35 ˚C respectively. These treatments, i.e., the exposure to different levels, i.e., high, and low, of light, moisture, and temperature, were applied to simulate the conditions that harvested bark is exposed to during the normal processes of harvesting, transport, and processing at the depot and/or factory, given that wattle bark can take up to four days to arrive at the extraction factories after harvesting (Havemann, 1992).

3.3.3 Sample preparation of treated bark

After the specified amount of treatment time, i.e., (t = 2 d and t = 4 d), the samples were weighed and then placed in a Virtis BT Pro series Freeze dryer at -70 ˚C and 175 mTorr for 48 hours until constant mass was achieved.

All the freeze-dried bark samples were milled to 0.5 millimetre (mm) using a ZM200 Retsch mill (Retsch, USA). The milled freeze-dried bark samples had a moisture content of less than 1 %. The samples were then stored at -20 ˚C for further analysis and to prevent sample degradation.

3.3.4 Factory bark sample collection and preparation

Chopped and chipped bark samples were collected individually from the three extract factories to determine whether NIRS could be used on these sample types. For both chopped and chipped bark, the particle size is set at 6 mm by 6 mm to allow for optimal extraction of tannins (Per’s comm, Tomlinson, 2022).

These samples were made up of harvested bark that had been delivered to the factories for the manufacture of wattle extract powders and solid extract. Bulk grab samples were taken periodically during the harvest season (from October 2020, until May 2021). The samples were taken by staff at the factories and stored in a freezer

and delivered to the ICFR under cold-chain conditions, after which they were freeze-dried immediately, and then stored in a freezer (-20 ˚C) to prevent sample degradation before further processing.

3.3.5 Tannin Extraction of milled freeze-dried bark

The milled freeze-dried bark was extracted using the Double Autoclave Water (DAW) (Avadianund Bridglall *et al*, 2025). The method is an enhance extraction technique typically using a sample mass of sample (+/- 5 g), that is extracted with distilled water (final volume of 500 ml). It uses two sequential autoclave cycles to efficiently extract water-soluble compounds, particularly tannins from the bark

3.3.6 UV Spectroscopy

The extract solutions were analysed by UV spectroscopy for the polyphenolic content, i.e*.,* tannins and other polyphenols, as an estimation of tannin content. The tannin levels were determined using the SLTC method (Section 3.3.7.1)

To determine the polyphenolic content, a 1 mL aliquot of extract solution was diluted in a 50 mL volumetric flask. An aliquot of the diluted extract solution was placed in a quartz cuvette and measured at 280 nm in the UV Spectrophotometer (Genova Nano Spectrophotometer Jenway, USA) with distilled water as a blank sample. The absorbance was measured, and the polyphenolic content was calculated as a percentage of the mass of the milled freeze-dried bark. Correlation analysis between polyphenolic % and tannin % using scatter plots, a paired T-test, and simple linear regression was done to determine whether polyphenolic content could reliably predict or indicate tannin content in bark extracts.

3.3.7 Reference chemistry analysis of bark

The extract solutions were analysed via the SLTC method. The samples were also analysed for macronutrients and micronutrients on an MP-AES 4100u (Agilent, USA), and total organic and inorganic Carbon (C), Nitrogen (N), and Sulphur (S) were determined using a Leco Trumac CNS Analyser (Leco, USA). This wet chemistry analysis was conducted as reference methods for the development of NIRS models, with the resulting analytical values used for calibration and validation datasets, as well as for independent test sets.

3.3.7.1 SLTC analysis

The wattle bark extract solution was subjected to SLTC analysis, i.e., extractives and tannin content analysis (Method SLT 2/3e), non-tannin content (Method SLT 2/3d), total soluble content (Method SLT 2/3c), total insoluble content (Method SLT 2/3f), and colour assessment (Method SLT 2/3g), as described in the standard leather tanning SLTC methods (Leather Technologists Pocket Book, Leafe, 1999). The extractive content of wattle bark is calculated by determining the dry mass of the extraction solution. For the determination of total soluble content, the extract solution is filtered through two layers of Whatman GF/C glass filter paper (Merck, Germany), and the total soluble content is determined from the dry mass of the filtered solution. The insoluble content is determined by the difference between the extractives and the total soluble extractives content. The non-tannin content is determined using commercial animal hide powder (BLC Leather Technology, USA). The hide powder is chemically prepared before analysis by the addition of a 3 % chrome solution (SLTC method number SLT 3). The extraction solution is added to the treated hide powder. The tannins in the extract solution bind to the proteins in the hide powder. The non-tannin content is left behind in the extract solution and is determined by the dry mass of the solution.

The tannin content is calculated as per the following formula:

> % Tannin = % Extractives – (% Non-Tannin + % Insoluble).

The constituents are expressed as a percentage of the dry bark. The red and yellow colour of the bark extract solution is determined within 30 minutes of extraction. The colour is determined in a 10 mm cell on a manual Model E Lovibond Tintometer (Lovibond, England). An aliquot of extraction solution is filtered through Whatman GF/C filter pads. The corrected Lovibond colour value is calculated as per the following formula:

> Corrected Lovibond Colour of bark = (Lovibond colour x aliquot of solution filtered x extractives % ÷ tannin % ÷ volume of extraction solution ÷ mass of dry bark)

Lovibond colour analysis does not use formal SI units (Gibson,1927), therefore in this work, it was reported as follows:

Lovibond Red = LRU

Lovibond Yellow = LYU

3.3.7.2 Quantitative analysis of bark for Macronutrients and Micronutrients

Single sample analysis was carried out, as one gram of freeze–dried, milled, and homogenized bark was reduced to ash in a muffle furnace (Nabertherm, China) at 500 °C for four hours. The ash was then digested by gentle heating on a hotplate in 5 mL of 16 % hydrochloric acid in silica crucibles. The digested samples were filtered through pre-wetted Whatman no. 42 filter paper (Merck, Germany) and made up to 50 mL with deionized water in a volumetric flask, for further analysis.

Mixed standard solutions were prepared from certified reference standards (De Bruyn Spectroscopic Solutions, South Africa) for Aluminum (Al), Boron (B), Calcium (Ca), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Phosphorus (P), and Zinc (Zn). Samples were analyzed on an Agilent 4100 Microwave Plasma Atomic Emission Spectrometer (MP-AES) (Agilent, USA). Standard reference curves and results were reported in mg kg^{-1}.

3.3.7.3 Analysis of total CNS

The analysis of total organic and inorganic C, N, and S was performed on a Leco Trumac CNS analyser (Leco, USA) from ~0.2 g of bark material.

3.3.8 NITS and NIRS analysis of bark extract solutions and milled freeze-dried bark powder

The bark extract solutions, and the freeze-dried and milled bark (fresh, treated, and factory bark) were scanned in triplicate on a Bruker MPA FT-NIR spectrometer (Bruker, USA) at a constant temperature of 20 ˚C.

3.3.8.1 NITS and NIRS spectral acquisition of bark extract solutions

The bark extract solution spectra were collected from samples placed in a transmission cell using transmission mode, as well as using a transflectance stamp using the reflectance mode on the integrating sphere module of the Bruker MPA FT-NIR instrument. The spectra were captured using the OPUS LAB software (version 6.2, Bruker, Germany) in the spectral range from 14963 cm^{-1} to 3574 cm^{-1} at a resolution of 8 cm^{-1}. The processes for using the transmission cell and transflectance stamp are described below:

Transmission cell: 20 bark extract solutions were placed in a 10 mm cuvette, and transmittance spectra were acquired in triplicate using the transmission cell i.e., NITS. Ten bark quality parameters were determined using this method.

Transflectance Stamp: 20 bark extract solutions were placed in 22 mm vials with a stainless steel 2 mm path length transflectance stamp. The samples were measured by reflectance spectroscopy in triplicate, i.e., NIRS. Ten bark quality parameters were determined using this method.

3.3.8.2 NIRS on milled freeze-dried bark powder

The samples were placed in a 20 mm diameter quartz vial using reflectance mode on the integrating sphere on the Bruker MPA FT-NIR instrument in the spectral range from 14963 cm^{-1} to 3574 cm^{-1} at a resolution of 8 cm^{-1}. The same sample was shaken after each scan and scanned three times. Each scan was treated as a single sample.

3.3.8.3 Model Development

The optimal spectral ranges for both NITS and NIRS were determined. Data pre-processing was performed using Bruker software (OPUS Quant), which offers three pre-processing options: NIR, General A, and General B (Miller, 2021).

Table 3.1 The three pre-processing steps available in OPUS for spectral data

Pre-processing Options	Key Features	Samples best suited for
NIR	[1]MSC/[2]SNV, 1st Derivative, Smoothing, Detrending	Samples with significant scatter and baseline variations
General A	1st Derivative, Light Smoothing	Homogeneous samples with minimal scatter
General B	2nd Derivative, Stronger Smoothing, Possible [1]MSC/[2]SNV	Complex or noisy samples with overlapping features

[1]Multiplicative Scatter Correction

[2]Standard Normal Variate

General B was found to yield the lowest Root Mean Squared Error of Prediction (RMSEP) and was therefore selected as the preferred method. Optimisation for General B pre-processing was further validated, as it resulted in the lowest Root

Mean Squared Error of Cross-Validation (RMSECV) and achieved the highest ranking in the optimisation step within the OPUS program. Optimisation is the process of trying different preprocessing + wavelength ranges + Partial Least Squares Regression (PLSR) factor numbers and select the combination that gives the lowest validated error (RMSECV/RMSEP) with minimal bias.

For both NITS and NIRS, the sample parameter values, and corresponding spectra were utilised in the OPUS Quant software (version 8.5, Bruker, Germany) to develop calibration and validation models using PLSR. However, this work focuses solely on the validation models, with the calibration models provided as supplementary data files.

3.3.8.4 Data pre-processing, model optimisation, development, and validation of NIRS models

Data processing was performed in the OPUS QUANT software environment spectra using PSLR and associated bark quality parameters data (SLTC and quantitative elemental analysis) were split randomly using software, into calibration (70 % of samples) and validation (30 % of samples) datasets to develop a prediction model. Validation model performance was recorded as an indicator of model performance. The model performance for each parameter, the informative spectral ranges, R^2, RMSEP, and RPD values were recorded to identify the best possible models. The RPIQ was used as a final step to evaluate the model performance and was calculated by dividing the interquartile range (IQR) of the reference values by the RMSEP to measure model prediction performance. Both RPD and RPIQ was used to screen model performance because , RPD was used to screen the model performance whereas RPIQ provides a more robust final evaluation by reducing the influence of outliers.

The development of NIRS methods directly from milled freeze-dried bark was broken into three experiments with different bark treatments, to explore the effects of material variation on model development, as listed below:

Experiment Three: 20 fresh (untreated), freeze-dried, and milled bark samples were scanned in triplicate. NIR pre-processing was used for model optimisation. The NIRS fresh bark model was developed and can now be used to predict 26 bark quality parameters using the OPUS Quant Analysis software.

Experiment Four: 70 treated (aged), freeze-dried, and milled bark samples were scanned in triplicate. General B pre-processing was used for model optimisation. The eight important bark quality parameters were determined using this method.

Experiment Five: 20 samples of chipped factory bark that had been freeze-dried and milled were scanned in triplicate. NIR pre-processing was used for model optimisation. Prediction of 21 bark quality parameter can be done in the OPUS Quant Analysis software.

Once an optimal model was developed for each parameter, it was used to predict values for an independent test set. The independent test set consisted of samples that had not been included in the model development and had reference chemistry analysis performed separately to obtain true values for comparison with NIRS predicted values. The number of samples used for the independent test set was between 10 % and 20 % of the sample size that was initially used to create the various models. The accuracy of these predictions was then used to determine the accuracy of the prediction models compared to the reference chemistry results. The independent test set was analysed in the Quant Analysis module of the OPUS software package (version 8.5, Bruker, Germany), where the sample spectra were loaded along with the methods containing the prediction models for each of the parameters.

An independent test set was created for Experiments Three, Four, and Five on the bark quality parameters used by the factories for assessment (i.e., extractives %, tannin %, non–tannin %, insoluble %, Lovibond red, and Lovibond yellow). The results were compared statistically to evaluate the predictive performance of the model. The model performance was measured in terms of correlation and comparison with true values for the independent test set. Scatter plots with linear regression (where the x-axis is the independent variable (true value) and the y–axis is the dependent variable (predicted value)) and regression analysis were performed in Microsoft Excel to compare results.

3.4 Results

The milled freeze-dried bark samples had a moisture content of less than 1 % for all samples analysed in the experiments below.

3.4.1 UV Spectroscopy for polyphenolic content

The comparison between the UV spectroscopic and SLTC methods showed that UV spectroscopy was not able to predict tannin content. As shown in Figure 3.1 there was a low correlation between the polyphenolics % determined UV absorbance compared with the tannin % content determined using the SLTC methods (R^2 = 0.09). The relationship is likely not statistically significant, given the weak slope and scatter.

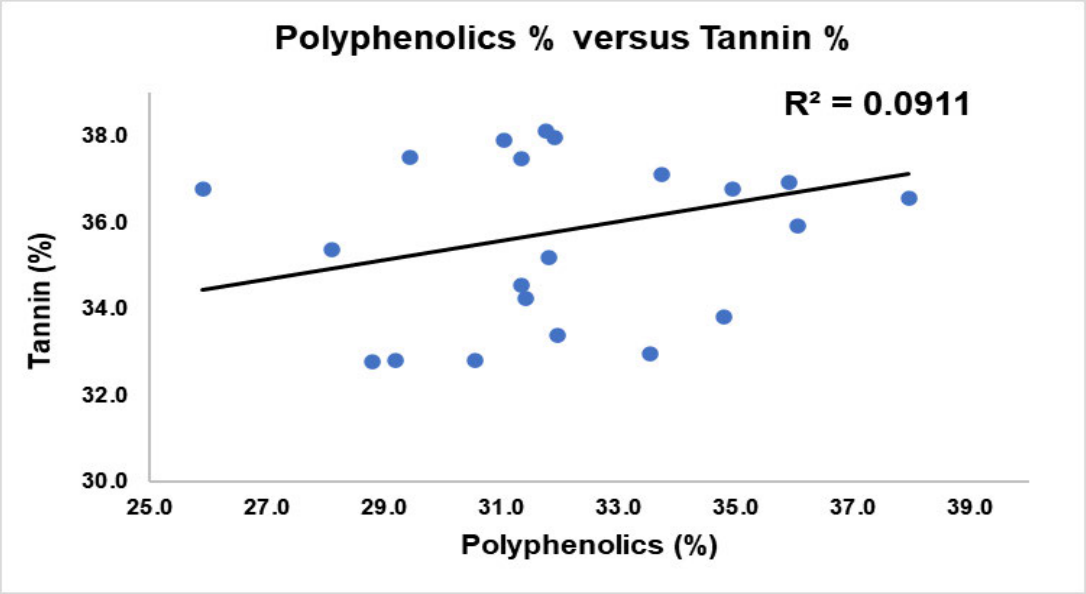


Figure 3.1 A scatter plot comparing UV-determined Polyphenolics % with SLTC-determined Tannin %. The solid trendline shows a weak correlation between the two variables.

The paired t-test indicated that the level of polyphenolics (%) was significantly lower than the level of tannins (%) (p-value < 0.001). The variance for the polyphenolics % was also much higher than the tannins % as shown in Table 3.2.

Table 3.2 The results of a paired t-test for polyphenolics % (UV) versus tannins % (SLTC)

	Polyphenolics %	**Tannins %**
Mean	31.4	35.6
Variance	21.8	3.0
p (T<=t) one-tail	< 0.001	

3.4.2 NITS and NIRS validation model performance

3.4.2.1 NITS of bark extract solution

The NITS performance model for the transmission cell method of spectral capture had R^2 values of 70 % and higher for several parameters, as shown in Table 3.3. Although the RPIQ values were regarded as good, due to the Lovibond Red having a low R^2 and RPD, the models generated using NITS were not tested further with independent test samples.

Table 3.3 NITS validation model performance for 10 bark quality parameters on bark extract solutions using a transmission cell for measurement.

Parameter	Unit	Range (Min)	Range (Max)	Spectral Range	R^2	RMSEP	RPD	IQR	RPIQ
Extractives	%	47.1	53.6	9947.7 - 7394.2	71.24	0.88	1.87	6.5	7.4
Tannin	%	30.5	41.3	8246.6 - 7394.2	88.74	0.72	3.29	10.8	15.0
Non-Tannin	%	10.2	15.6	9099.1 - 7394.2	84.11	0.56	2.51	5.4	9.6
Insoluble	%	1.1	3.4	10796.2 - 8246.6	79.81	0.26	2.23	2.3	8.8
Tannin/Non-Tannin Ratio		2.1	3.9	9091- 7394.2	73.78	0.21	1.96	1.8	8.6
Polyphenolics	%	25.9	38.0	11644.8 - 10792.2 & 9947.7 - 7394.2	67.15	2.82	1.77	12.1	4.3
Lovibond Red	LRU	0.9	1.8	12493.4 - 11641	53.54	0.19	1.47	0.9	4.7
Lovibond Yellow	LYU	1.6	3.2	9091- 7394.2	63.20	0.24	1.65	1.6	6.7
Turbidity	NTU	201	371	8246 - 7394.2	54.11	28.20	1.48	170	6.0
pH	pH units	4.8	6.0	10796.2 - 7394.2	66.81	0.13	1.75	1.2	9.2

Table 3.4 shows the performance data for several wattle bark parameters. The models were not considered for further testing or evaluation due to their relatively poor performance, moderate R^2 values and the RPIQ of 0.9 for tannin % which shows low model performance.

Table 3.4 NITS validation model performance on 10 bark quality parameters for bark extract solutions using a transflectance stamp for measurement.

Parameter	Unit	Range (Min)	Range (Max)	Spectral Range	R^2	RMSEP	RPD	IQR	RPIQ
Extractives	%	47.04	53.55	7151.1 - 4242.8	54.84	1.11	1.54	6.5	5.9
Tannin	%	30.51	41.31	6102 - 4597.7	13.66	12.00	1.08	10.8	0.9
Non-Tannin	%	10.17	15.60	6102 - 4242.8	57.07	0.91	1.53	5.4	8.0
Insoluble	%	1.06	3.38	7151.1 - 4242.8	22.89	0.51	1.14	2.3	4.5
Tannin/Non-Tannin Ratio		2.09	3.85	6102 - 5446.3 & 4605.4 - 4242.8	40.02	0.31	1.30	1.8	5.7
Polyphenolics	%	25.9	37.95	6102 - 4597.7	58.26	3.18	1.57	12.1	3.8
Lovibond Red	LRU	0.91	1.83	4428 - 4242.8	34.15	0.23	1.23	0.9	4.0
Lovibond Yellow	LYU	1.62	3.18	7151.1 - 6618.8 & 5454 - 4242.8	51.75	0.28	1.47	1.6	5.6
Turbidity	NTU	201	371	7151.1 - 4242.8	80.09	18.60	2.24	170	9.1
pH	pH units	4.78	5.97	6102 - 5446.3 & 4428 - 4242.8	39.22	0.18	1.28	1.9	6.6

3.4.2.2 NIRS validation models for milled freeze-dried bark

NIRS models were created from NIR spectra collected directly from milled freeze-dried bark for Experiments Three, Four, and Five, i.e., without performing an extraction. Reference data was produced using DAW extractions and SLTC analysis of the extract solutions.

Experiment Three: The NIRS model for fresh bark, i.e., bark that had been collected from the trees in the field and processed immediately, worked extremely well with 79 % of the models having R^2 values above 90 %, 14 % above 80 %, and 7% above 70 %, as shown in Table 3.5. The RPD was above 2 for all 26 bark quality parameters tested, which shows that the NIRS models developed could accurately predict the levels of the parameters tested. The RPIQ values exceeded 2.5 for all quality parameters of the model, indicating strong model robustness and reliable predictive performance.

Table 3.5 NIRS validation model performance of 26 bark quality parameters for freshly collected and processed milled freeze-dried bark.

Parameter	Unit	Range (Min)	Range (Max)	Spectral Range	R^2	RMSEP	RPD	IQR	RPIQ
Freeze-dried Moisture	%	37.1	48.1	7234.7 -4242.8	96.73	0.53	5.67	11.0	20.8
Diameter at Breast Height (DBH)	cm	10.6	16.9	6102 -4242.8	94.48	0.43	4.31	6.3	14.7
Extractives	%	47.04	53.55	7234.7 -4242.8	92.40	0.49	3.82	6.5	13.3
Tannin	%	30.51	41.31	7454- 4242.8	94.25	0.60	4.21	10.8	18.0
Non-Tannin	%	10.17	15.60	6102 -4242.8	92.72	0.37	4.05	5.4	14.7
Insoluble	%	1.06	3.38	6102.0 - 4242.8	94.79	0.13	4.52	2.3	17.8
Tannin/Non-Tannin Ratio		2.09	3.85	6672.8 - 6094.3	93.26	0.20	4.03	1.8	8.8
Polyphenolics	%	25.9	37.95	5454.0 - 4242.8	96.56	0.92	5.43	12.1	13.1
Lovibond Red	LRU	0.91	1.83	7234.7 - 4242.8	92.98	0.08	3.79	0.9	11.5
Lovibond Yellow	LYU	1.62	3.18	7234.7 - 4242.8	94.40	0.10	4.24	1.6	15.6
Turbidity	NTU	201	371	6102.0 - 4597.7	94.34	10.30	4.23	170	16.5
pH	pH units	4.78	5.97	5454.0 - 4242.8	93.35	0.05	3.89	1.2	23.8
Al	mg kg^{-1}	74.23	736	7234.7 - 4242.8	94.97	35.50	4.59	662	18.6
B	mg kg^{-1}	8.07	11.19	5454.0 - 4597.7	88.31	0.30	3.00	3.2	10.4
Ca	mg kg^{-1}	6970	13044	7234.7 - 4242.8	92.65	43.50	3.71	6074	140
Cu	mg kg^{-1}	0.87	2.96	7234.7 - 4242.8	94.86	0.13	4.51	2.1	16.1
Fe	mg kg^{-1}	45.70	505	7234.7 - 4242.8	95.42	23.10	4.77	459	19.9
K	mg kg^{-1}	1325	3450	7234.7 - 4597.7	88.15	201.00	2.91	2126	10.6
Mg	mg kg^{-1}	497	1280	7234.7 - 4242.8	87.97	84.50	3.06	783	9.3
Mn	mg kg^{-1}	25.04	60.15	7234.7 - 4242.8	93.67	1.96	4.84	35.1	17.9
Na	mg kg^{-1}	60.91	259	7234.7 - 4242.8	75.07	27.10	2.01	198	7.3
P	mg kg^{-1}	0	228	7234.7 - 6094.3	86.93	25.00	2.87	228	9.1
Zn	mg kg^{-1}	0	8.80	7234.7 - 4242.8	78.55	1.06	2.16	8.8	8.3
C	%	47.06	50.86	7234.7 - 4242.8	90.64	0.25	3.40	38.0	15.1
N	%	0.78	1.15	6102.0 - 4242.8	95.68	0.02	4.81	0.4	18.5
S	%	0.02	0.06	7234.7 - 6094.3 & 5454.0 -4242.8	96.17	<0.01	5.12	0.03	4.0

The R^2 values of the scatter plots using only three samples indicated a good to strong correlation between true and predicted values for the bark quality parameters, as shown in Figure 3.2 (A – F). However, the regression p-values did not show a significant correlation. However, this evaluation was limited due to only 3 data points.

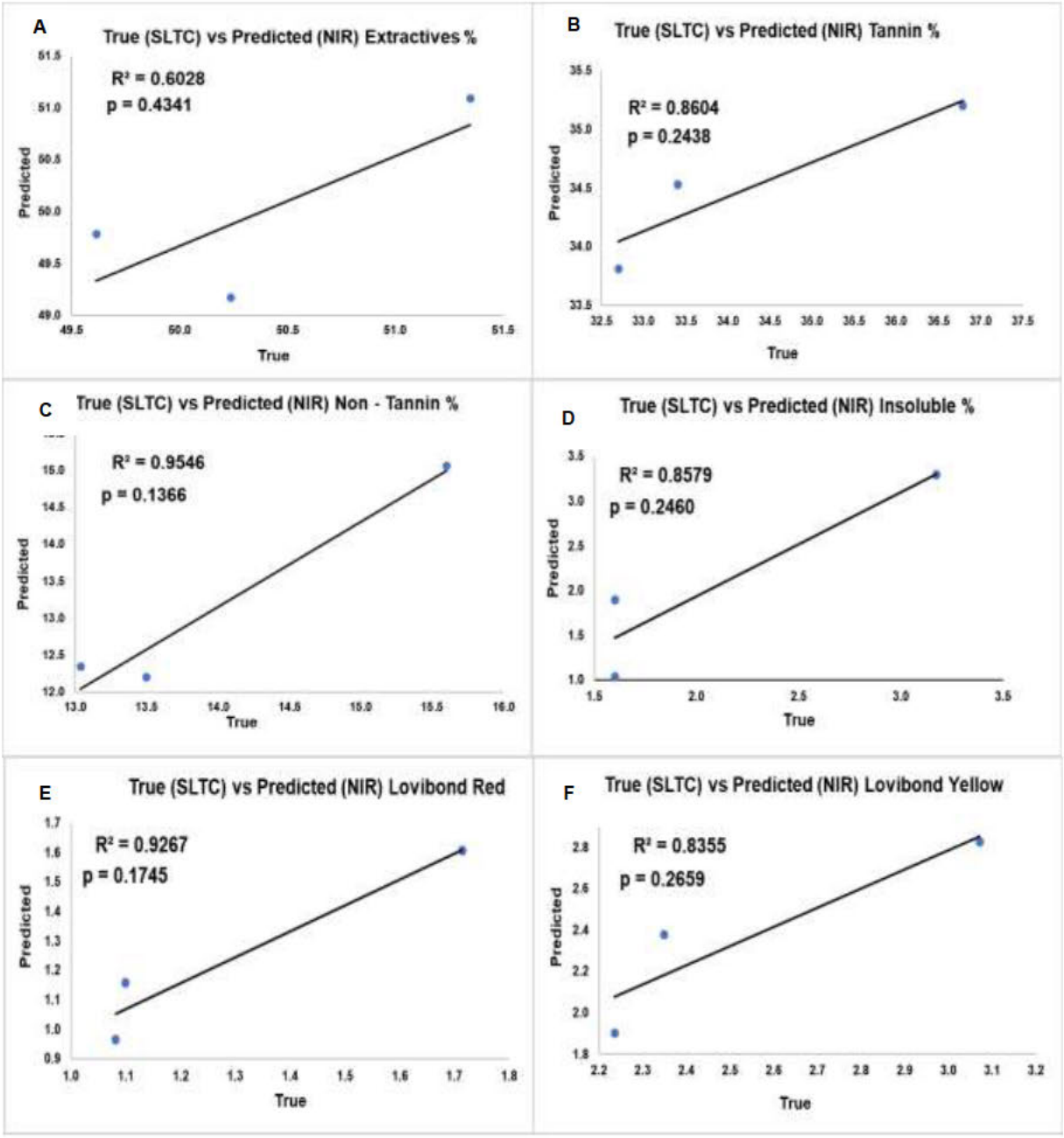


Figure 3.2 Scatter plots for the independent set of NIRS (fresh bark) versus reference data for (A) Extractives %, (B) Tannin %, (C) Non -Tannin %, (D) Insoluble %, (E) Lovibond Red (LYR), and (F) Lovibond Yellow (LYU).

Experiment Four: The NIRS predictive model for the treated bark, i.e., aged, wetted/unwetted, and light exposure, performed reasonably well as shown in Table 3.6. The Lovibond Red, Tannin, Extractives had high R^2, low RMSEP, high RPD and RPIQ indicating the good model predictive performance.

Table 3.6 NIRS validation model performance for treated (aged, wet/dry, high/low temperature, and light/dark exposure) freeze-dried, and milled bark. The model was created to determine the eight priority bark quality parameters.

Parameter	Unit	Range (Min)	Range (Max)	Spectral Range	R^2	RMSEP	RPD	IQR	RPIQ
Extractives	%	43.7	55.8	5338.3 - 4381.7 & 3911.1 - 3749.1	73.28	0.64	1.95	12.1	18.9
Tannin	%	26.6	41.3	5338.3 - 3749.1	80.64	0.82	2.29	14.7	17.9
Non-Tannin	%	10.6	18.9	5338.3 - 4219.7	65.70	0.95	1.71	8.3	8.7
Insoluble	%	0.9	3.5	5338.3 - 3904.3	17.14	0.61	1.10	2.6	4.3
Tannin/Non-Tannin Ratio		1.2	3.3	5338.3 - 4543.7	64.87	0.23	1.23	2.1	9.1
Manual Lovibond Red	LRU	0.8	3.0	5338.3 - 3749.1	89.76	0.10	3.16	2.2	22.0
Manual Lovibond Yellow	LYU	1.5	5.8	5338.3 - 4543.7	81.56	0.25	2.16	4.3	17.2
Turbidity		119	371	5184.0 - 3749.1	71.88	11.9	1.92	252	21.2

The correlation of true versus predicted values of eight samples tested for the major quality parameters was good for the tannin %, Lovibond Red, and Lovibond Yellow because the R^2 was greater than 70% with a significant correlation between the NIRS model and reference chemistry ($p < 0.05$). However, the Extractives %, Non-tannin %, and Insoluble % showed poor correlations, low R^2 values, and correlations with the reference chemistry results were not significant ($p > 0.05$), as shown in Figure 3.3 (A – F).

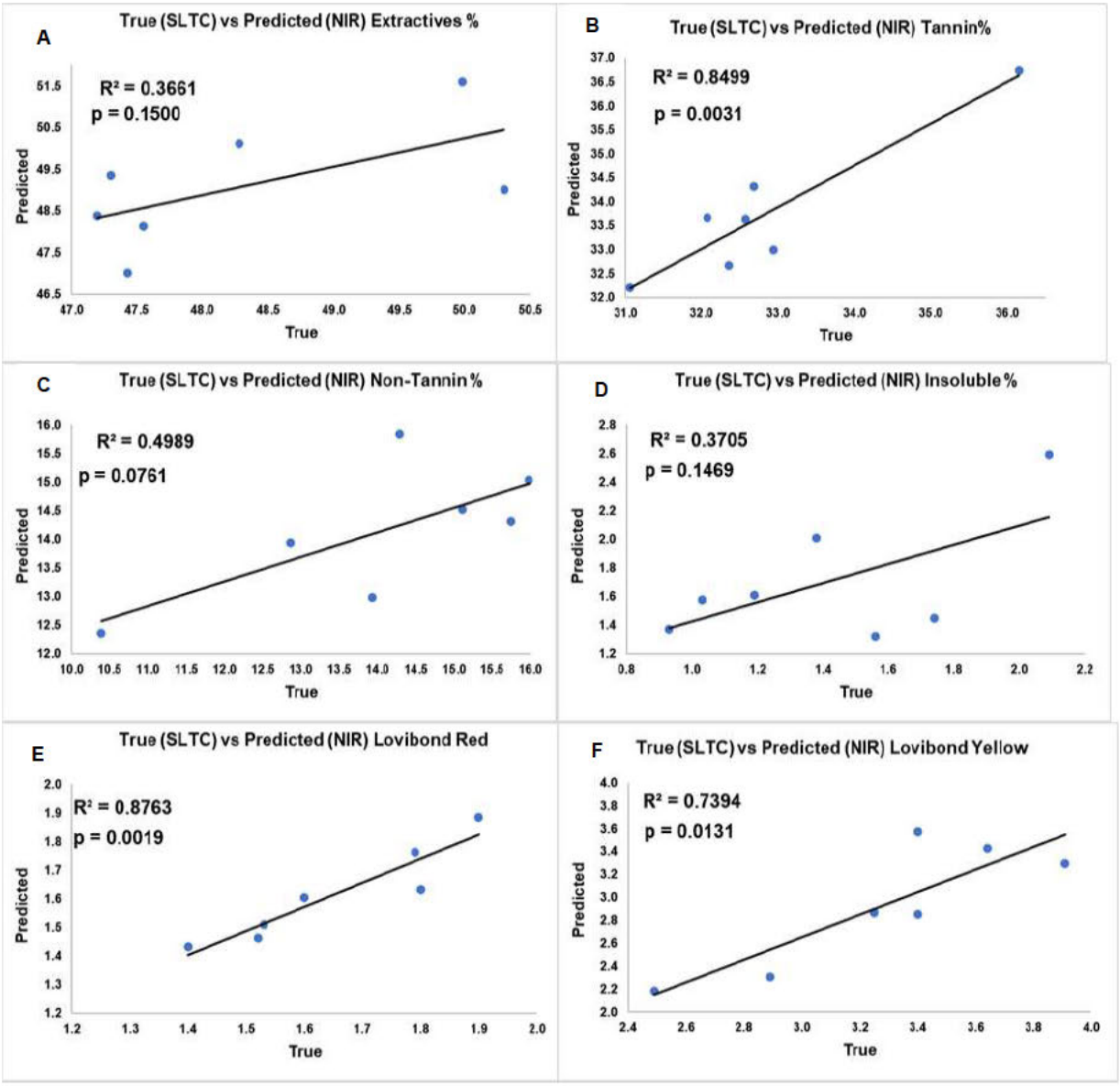


Figure 3.3 Scatter plots for the independent set of NIRS (treated bark) versus reference data for (A) Extractives %, (B) Tannin %, (C) Non -Tannin %, (D) Insoluble %, (E) Lovibond Red (LYR), and (F) Lovibond Yellow (LYU).

Experiment Five: The NIRS models developed for the milled, freeze-dried factory chipped produced good models, with more than 60% of the models having R^2 values greater than 90%. The validation model performances for all parameters are shown in Table 3.7. The models were created using 25 bark samples. The RPDs and RPIQ showed the model is reliable for key chemical and colorimetric parameters such as Tannin % and Lovibond colour (red and yellow).

Table 3.7 NIRS validation model performance for chipped and chopped factory milled freeze–dried bark. The model was created for 21 bark quality parameters.

Parameter	Unit	Range (Min)	Range (Max)	Spectral Range	R^2	RMSEP	RPD	IQR	RPIQ
Extractives	%	44.3	59.1	7506 - 6094.3 & 5454 -4242.8	98.26	0.30	7.56	14.8	49.3
Tannin	%	32.6	37.4	7506 - 4242.8	99.73	0.05	19.20	4.8	96.0
Non-Tannin	%	10.7	16.6	6102 - 4242.8	98.06	0.35	7.21	5.9	16.9
Insoluble	%	0.6	1.7	6102 - 4242.8	75.09	0.14	2.04	1.1	7.9
Tannin/Non-Tannin Ratio		1.5	3.5	5454 - 4242.8	97.80	0.06	6.85	2.0	33.3
Manual Lovibond Red		0.8	2.2	7506 - 6094.3 & 5454 - 4242.8	99.58	0.02	17.60	1.4	70.0
Manual Lovibond Yellow		1.3	4.5	7506 - 4242.8	99.29	0.05	11.90	3.2	64.0
Al	mg kg^{-1}	57.4	1506	7506 - 4242.8	86.39	121.00	2.76	1449	12.0
B	mg kg^{-1}	6.9	12.3	7506 - 4242.8	96.11	0.29	5.09	5.4	18.6
Ca	mg kg^{-1}	3637	11749	7506 - 4242.8	86.15	822.00	2.71	8111	9.9
Cu	mg kg^{-1}	0.9	5.0	9303.7 - 4242.8	95.87	0.18	5.43	4.1	22.8
Fe	mg kg^{-1}	47.01	1115.6	7506 - 4242.8	88.52	68.70	2.98	1069	15.6
K	mg kg^{-1}	2274	5422	6102 - 4242.8	87.61	251.00	2.99	3148	12.5
Mg	mg kg^{-1}	673	1576	9403.7 - 4597.7	90.61	55.50	3.27	902	16.3
Mn	mg kg^{-1}	17.1	113.1	7506 - 4242.8	89.59	38.30	3.21	96.0	2.5
Na	mg kg^{-1}	39.2	534	7506 - 4242.8	92.06	21.30	3.57	495	23.2
P	mg kg^{-1}	0	264	7506 - 4242.8	92.81	19.60	3.75	264	13.5
Zn	mg kg^{-1}	0	4.9	9403.7 - 4597.7	75.88	0.55	2.04	4.9	8.9
C	%	47.3	50.8	6102 - 4242.8	92.29	0.25	3.73	3.5	14.0
N	%	0.8	1.2	7506 - 6094.3 & 5454 - 4242.8	96.95	0.02	5.99	0.4	20.0
S	%	0	0.1	7506 - 4242.8	86.77	0.01	2.75	0.1	10.0

The correlations between predicted results and the independent test sample results for the factory bark material were greater than 0.9 for all parameters (except tannin% 0.85) and these correlations were significant ($p<0.05$) as shown in Figure 3.4 (A – F).

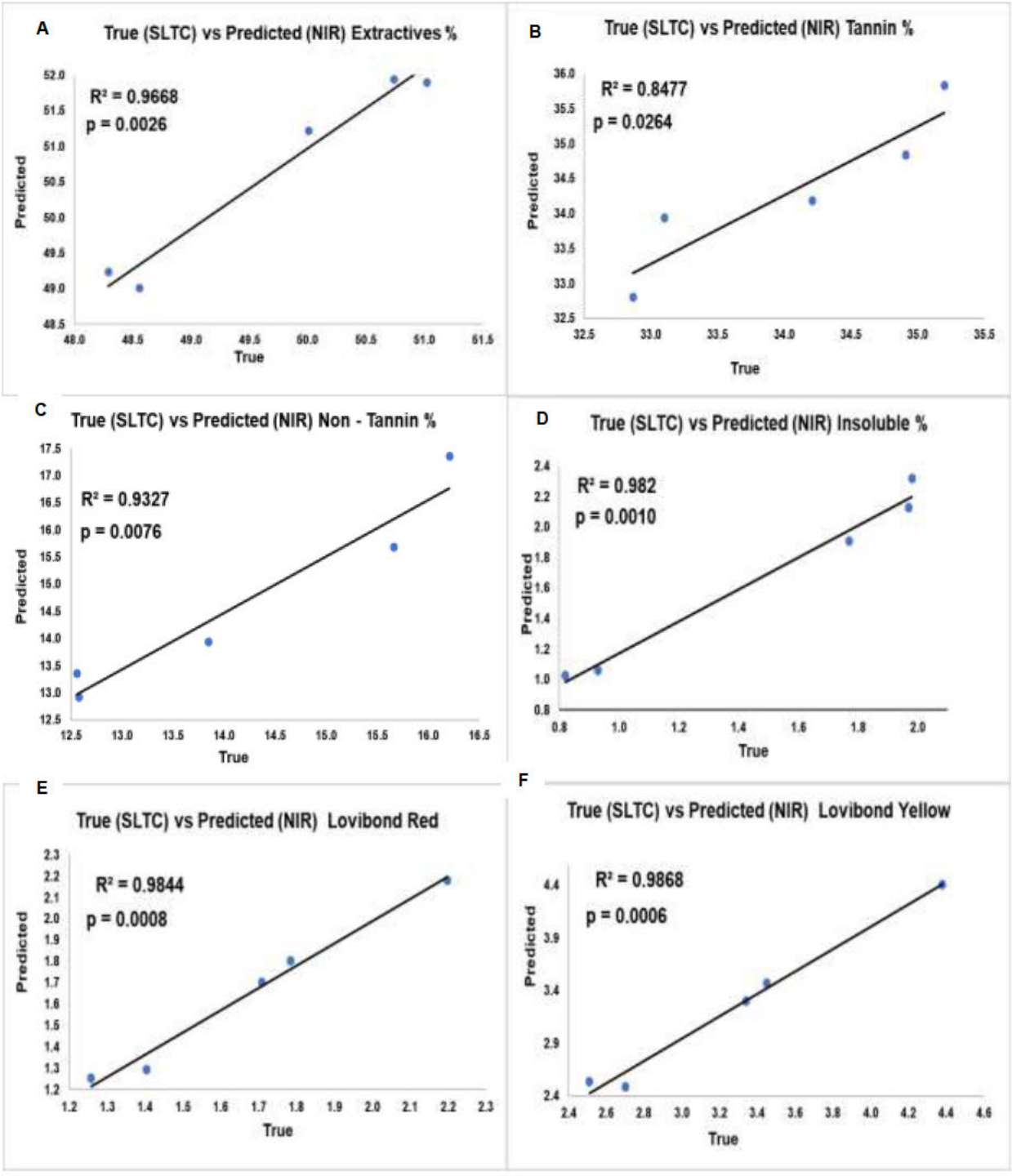


Figure 3.4 Scatter plots for the independent set of NIRS (factory bark) versus reference data for (A) Extractives, (B) Tannin, (C) Non -Tannin, (D) Insoluble, (E) Lovibond Red (LRU), and (F) Lovibond Yellow (LYU).

3.5 Discussion

The SLTC method for tannin analysis is a tedious, complex, and slow process that takes up to three days for a single sample. The method is also expensive because the hide powder used for tannin analysis costs an average of € 275 (+/- R6500 at current exchange rates (April 2025)) per tin due to import costs from BLC. Only 60 samples can be analysed using hide powder from a single tin. It also has a high error level, with up to 2 % absolute error being considered acceptable, using different analysts in a single laboratory, according to the official SLTC methods (Garbutt and Nobel, 1983). Results compared between different laboratories can have greater variation (Hillis, 1997). Therefore, it would be beneficial if an alternative reliable, rapid, and cost-effective method of tannin analysis could be developed to either replace the hide powder method or provide a high-throughput screening method for quality control.

An experiment to test whether UV Spectroscopy could be used to determine the polyphenolic content and indirectly estimate the tannin % showed a poor correlation between the two sets of data, indicating that this method was not suitable for predicting SLTC-based tannin levels. The UV spectroscopy results were significantly different from those determined by SLTC methods ($p < 0.05$) and the polyphenolic estimation also had a high variation in results obtained. It has been previously suggested a close relationship exists between tannin and polyphenolic percentage, which can be rapidly estimated using UV spectroscopy in extract powder samples (Roux, 1951). However, the bark samples are unrefined in comparison to extract powder. The turbidity of the extract solution influences the results because suspended particles cause light-scattering effects (Lourenco *et al.,* 2012). Therefore, due to the inconsistent composition of the bark extract solution (which can be influenced by the environment (Abbas *et al.,* 2017)), the UV spectroscopy method was found to be inadequate for the rapid determination of the tannin content of bark extract solutions. It is also unlikely to provide colour information.

On the other hand, NIRS has been shown in several studies to be able to estimate tannin content rapidly and inexpensively in plant material (Donkin and Pearce, 1995, Grasel and Ferrão, 2016, Menezes *et al.,* 2014, Nascimento *et al.,* 2022) Donkin and Pearce (1995) showed that NIRS was able to predict the levels of tannins, non-

tannins, and extractives. However, Donkin and Pearce (1995) argued that NIRS could not replace the SLTC as it is dependent on the original SLTC "golden standard" wet chemistry methods. On the other hand, with advances in both instrument and computing technology NIRS has become a mainstream technique used in many industries such as grain and dairy production, although models are regularly updated using reference methods (Cozzolino, 2009) which reduces cost and turnaround times for sample analyses while providing reliable results (Williams et al., 2019). Therefore, this technology could be used to reduce the number and cost of SLTC analysis as an SLTC-derived NIRS method for bark or even extract powder analysis. To implement the use of NIRS as a testing method, SLTC could be run as a model quality control on a smaller percentage of samples to validate the NIRS results. The addition of reference standards and applying bias adjustments when necessary could lead to eventually having a model that would become robust enough to limit SLTC testing to a minimal amount.

The benefits of being able to apply NIRS technology in the wattle bark industry are substantial in terms of both analysis time and cost. It allows for numerous quality parameters to be determined from a single scan in which the absorbances across a range of wavelengths in the near-infrared region are captured. The research and development in this work has generated an optimal method of presenting the sample to the analyser to produce robust and accurate prediction models. A validation study would confirm the potential of these models to be used by the industry.

NIR transmission spectroscopy for process monitoring has also become increasingly popular in the refining, petrochemical, materials, and pharmaceutical industries (Jimaré Benito *et al.,* 2008). Therefore, the use of the transmission cell and transflectance stamp for the analysis of bark extract solutions was explored. The transmission cell had R^2 values between 70 % - 80 % and RPIQ values above the 2.5 most of the bark quality parameters such as extractive %, tannin %, non-tannin %, insoluble %, and tannin/ non-tannin ratio. The model performed poorly for colour (Lovibond Red and Lovibond Yellow) with low R^2 and RPD values. These quality parameters are of primary interest in this work. The transflectance stamp method had low performance indices which indicated average to poor model prediction which showed no further value to this work and was not considered further. The transmission cell had a path length of 1 mm through the sample, and the

transflectance stamp had a path length of 2mm through the sample. The latter method outperformed the first approach, which demonstrates the effect of water on prediction models in high moisture samples, as explained by Cozzolino *et al*. (2008). Jensen and Bak (2002) showed that NIRS instrument detectors become saturated when high levels of water are present, as is the case with the extract solution which has > 95 % water. Therefore, this approach was not investigated further because the analysis of dry bark with minimal processing would be more desirable and the steps involved in extraction would create more labour and potential for error, especially with high sample numbers. However, transmission cell approach could be considered for monitoring thin liquor (low solid bark extract solution), and thick liquor quality parameters during the factory extraction to monitor extraction efficiency, detect problems or to identify high-value product batches that can be isolated from lower quality ones. It could have potential for use on an instrument that does not have a transmission mode of capturing spectra, if liquid samples need to be analysed. However, if this was to be developed, a concentration step may be necessary to reduce the water content of samples (Guo *et al.,* 2023).

The development of NIRS models from the materials subjected to various bark treatments (fresh, age/moisture/light, and factory) showed great promise. This was due to the low moisture content of the samples (Kauppinen *et al.,* 2014). The performances were evaluated according to the correlations between true and predicted results as well as the ratio of the standard error of prediction to standard deviation (RPD) (Williams *et al.,* 2019). However, an independent test, also referred to as the evaluation or monitoring set, which tests the model, and its accuracy (Mancini *et al.,* 2019) would be the ultimate test of whether the models would be robust enough for future use on unknown samples. This was beyond the scope of this work but is encouraged for further investigation should it benefit the wattle bark industry.

The model for analysing fresh bark (Table 3.4) showed good R^2, RPD and RPIQ values for the validation models. However, while correlations were high, significance was low. This was due to low sample numbers (both for calibration and independent test set validation), which can be a source of poor model performance (Foley *et al.,* 1998). Another practical consideration is that bark collection and processing were performed under ideal conditions that do not represent the reality of the bark

harvesting and analysis process. However, developing a NIRS prediction model from such samples could be useful in future research and breeding for improved bark quality parameters because bark changes and quality would not be influenced by logistic or environmental factors. The use of NIRS analysis of oven-dried bark would speed up the process. However, this work showed that the oven drying process would cause darkening, possibly to completion, which would make colour prediction difficult. The changes to tannin chemistry, i.e., protein binding, may also be significant. Therefore, the capture of reflectance near-infrared spectra from freeze-dried bark powder is recommended, as suggested by Yazaki *et al.* (1993).

The simulation of ageing and environmental effects resulted in NIRS prediction models that performed well. However, the spectral ranges were different from those used to develop models for the fresh and factory material. The treatments affected the bark properties, which resulted in variations in the spectra. Table 3.5 shows that the spectral range included absorbances in the 5000 cm^{-1} region, and interference of the O-H bonds with water absorption occurs at this range. This results in a combination of C–H stretching vibrations and C–H deformation of the tannin molecule (Burns and Ciurczak, 2007). However, the models and the independent test set produced a good estimation of the tannin % and the Lovibond Red and Lovibond Yellow colours, which were the primary quality parameters investigated in this work. This shows that NIRS is suitable for future work investigating environmental parameters that affect those quality parameters. The extractives %, non-tannin %, and insoluble % models performed average due to low RPD (< 2). This was because the simulated ageing process altered the material chemical bonds that respond to near-infrared light in way that was different to the fresh and factory bark material. However, these models can still be used for estimation of these parameters as all of the RPIQ values were well above 2.5. It is worth noting that although it is possible that some of these properties could be derived mathematically from the tannin % calculation, this is work that could be explored in the future, if necessary, along with testing additional algorithms and data pre-processing techniques.

The models developed from SLTC analysed factory samples performed very well, and the independent sets showed that these models could be used for predicting

various bark quality parameters. The informative spectral regions used for both fresh and factory bark were similar, with most of the major parameters being determined from absorbances in the spectral range that includes 7100 cm^{-1}, which is associated with phenols (Skoog *et al.,* 2017). The factory bark models demonstrate that in addition to the typical SLTC-derived quality parameters, NIRS can be used by the industry to monitor macronutrients or micronutrients in the bark that may be contributing to the quality and quantity of bark products. This would also be valuable to growers for silviculture practices, and for researchers investigating the effect of different soils on bark extract quality. In addition, total carbon levels in the bark can be used in carbon credits or carbon tax estimations for forestry and bark factory operations (Muhammad *et al.,* 2022). This work clearly shows that NIRS is a suitable method for estimating and monitoring of bark quality from the plantation tree where it can be applied to research on-site quality and tree genetics, as well as in the factory to monitor incoming bark quality and increase factory production efficiencies and product quality.

The use of NIRS has many benefits as it involves no toxic chemicals, no waste generation, is non-destructive, has relatively few processing steps, and can be used to standardize analytical performance across various sites (Cozzolino, 2009). While benchtop instrument costs can be high, this can be recovered from the reduction in the use of expensive hide powder and labour costs. NIRS offers the wattle industry a modern technology for both research applications and quality assessment. Future research in lower-cost, portable NIRS units could also have many benefits to the wattle bark industry in South Africa.

3.6 References

Abbas, O., Compère, G., Larondelle, Y., Pompeu, D., Rogez, H. and Baeten, V. 2017. Phenolic compound explorer: A mid-infrared spectroscopy database. *Vibrational Spectroscopy*, *92*, 111-118.

Antoine, M.L., Simon, C. and Pizzi, A. 2004. UV spectrophotometric method for polyphenolic tannin analysis. *Journal of Applied Polymer Science*, *91*(4), 2729-2732.

Avadianund Bridglall, P., Laing, M., Naidoo, N., and Burgdorf, R. 2025. A novel laboratory method for the extraction of black wattle (*Acacia mearnsii* de Wild.) bark constituents. Journal of American Leather Chemists Association.120(10), pp 26 – 41.

Burns, D.A. and Ciurczak, E.W. 2007. *Handbook of Near-Infrared Analysis,* 3rd Edition. CRC Press, London.

Chan, J.M., Day, P., Feely, J., Thompson, R., Little, K.M. and Norris, C.H. 2015. *Acacia mearnsii* industry overview: current status, key research, and development issues. *Southern Forests: A Journal of Forest Science*, *77*(1),19-30.

Chang, S.K. and Zhang, Y. 2017. Protein analysis. *Food Analysis*, Volume 5, 315-331.

Cozzolino, D. 2009. Near infrared spectroscopy in natural products analysis. *Planta medica*, *75*(07), pp.746-756.

Cozzolino, D. and Morón, A. 2006. Potential of near-infrared reflectance spectroscopy and chemometrics to predict soil organic carbon fractions. *Soil and Tillage Research*, *85*(1-2), 78-85.

Cozzolino, D., Cynkar, W.U., Dambergs, R.G., Mercurio, M.D. and Smith, P.A. 2008. Measurement of condensed tannins and dry matter in red grape homogenates using near-infrared spectroscopy and partial least squares. *Journal of Agricultural and Food Chemistry*, *56*(17),7631-7636.

Cozzolino, D. 2009. Near-infrared spectroscopy in natural products analysis. *Planta Medica*, *75*(07), 746-756.

Donkin, M.J., Pearce, J., and Chetty, P.M. 1993. *Methods for Routine Plant Analysis in the ICFR Laboratories.* Bulletin Series 6/93, ICFR, Pietermaritzburg.

Foley, W.J., McIlwee, A., Lawler, I., Aragones, L., Woolnough, A.P., and Berding, N. 1998. Ecological applications of near-infrared reflectance spectroscopy–a tool for rapid, cost-effective prediction of the composition of plant and animal tissues and aspects of animal performance. *Oecologia*, *116*(3), 293-305.

Garbutt, D.C.F. and Noble, M.A. 1983. Diethylamino ethyl cellulose as a reagent in tannin analysis. *Report-Wattle Research Institute*. Pp 115 -120.

Germishuizen, I. 2022. Personal communication with Dr. I. Germishuizen. Research Director, ICFR, Pietermaritzburg, May 2022.

Gibson, K.S. 1927. *The Lovibond Color System:* A spectrophotometric analysis of the Lovibond glasses (No. 547). US Government Printing Office, Washington. Pp 4-7.

Gordon-Gray, C.G. 1953. A rapid method of determining the relative tannin content of young, living black wattle trees in genetic and breeding experiments. *Report-Wattle Research Institute*. Pp 42-48.

Grasel, F.S. and Ferrão, M.F. 2016. A rapid and non-invasive method for the classification of natural tannin extracts by near-infrared spectroscopy and PLS-DA. *Analytical Methods*, *8*(3), 644-649.

Gujrathi, A.M. and Babu, B.V. 2007. Environment-friendly products from black wattle. *Energy Education Science and Technology*, *19*(1), 37-44.

Guo, J., Huang, H., He, X., Cai, J., Zeng, Z., Ma, C., Lü, E., Shen, Q. and Liu, Y. 2023. Improving the detection accuracy of the nitrogen content of fresh tea leaves by combining FT-NIR with moisture removal method. *Food Chemistry*, *405*, p.134905.

Hagerman, A.E. and Butler, L.G. 1989. Choosing appropriate methods and standards for assaying tannin. *Journal of Chemical Ecology,* 15(6), 1795-1810.

Hagiopol, C. 2021. Natural polyphenols applications. In: *Natural Polyphenols from Wood: Tannin and Lignin–An Industrial Perspective*. K. Cheng and C. Hagiopol (Eds). Elsevier. pp 259.

Havemann, D.A. 1992. *The Manufacture and Uses of Wattle Bark Extract.* Ph.D. dissertation, University of Stellenbosch. Pp 31-48.

Hemingway, R.W. and Karchesy, J.J. 2012. *Chemistry and Significance of Condensed Tannins*. Springer Science & Business Media, London. Pp 517-527.

Hillis, W.E. 1997. Tannin chemistry. In: *Black Wattle and its Utilisation*. Rural Industries Research and Development Corporation, Canberra, Australia. pp*106*.

Jensen, P.S. and Bak, J. 2002. Near-infrared transmission spectroscopy of aqueous solutions: Influence of optical path length on the signal-to-noise ratio. *Applied Spectroscopy*, *56*(12), 1600-1606.

Jimaré Benito, M.T., Bosch Ojeda, C. and Sanchez Rojas, F. 2008. Process analytical chemistry: applications of near-infrared spectrometry in environmental and food analysis: an overview. *Applied Spectroscopy Reviews*, *43*(5), 452-484.

Kauppinen, A., Toiviainen, M., Lehtonen, M., Järvinen, K., Paaso, J., Juuti, M. and Ketolainen, J. 2014. Validation of a multipoint near-infrared spectroscopy method for in-line moisture content analysis during freeze-drying. *Journal of Pharmaceutical and Biomedical Analysis*, *95*, 229-237.

Lawrence, I. and Lin, K. 1989. A concordance correlation coefficient to evaluate reproducibility. *Biometrics*, 5(1), 255-268.

Leafe, M.K. (Ed.). 1999. *Leather Technologist's Pocketbook.* Society of Leather Technologists and Chemists, United Kingdom 227 -272.

Lourenco, N.D., Lopes, J.A., Almeida, C.F., Sarraguça, M.C. and Pinheiro, H. 2012. Bioreactor monitoring with spectroscopy and chemometrics: A Review. *Analytical and Bioanalytical Chemistry*, *404*(4), 1211-1237.

Mancini, M., Taavitsainen, V.M. and Toscano, G. 2019. Comparison of three different classification methods' performance for the determination of biofuel quality by means of NIR spectroscopy. *Journal of Chemometrics*, *33*(7), 3145.

Menezes, C.M., da Costa, A.B., Renner, R.R., Bastos, L.F., Ferrão, M.F. and Dressler, V.L. 2014. Direct determination of tannins in *Acacia mearnsii* bark using near-infrared spectroscopy. *Analytical Methods*, *6*(20), 8299-8305.

Miller, C.E. and Igne, B. 2021. Preprocessing Methods in NIR Spectroscopy. *Handbook of near-infrared analysis*, p.289.

Muhammad, M., Ratajczak, T., Watson, N., Zhu, J. and Little, P. 2022. Carbon footprint reduction by utilizing real-time NIR spectroscopy for RVP measurement in natural gas condensate stabilizers. In *International Petroleum Technology Conference,* OnePetro.

Nascimento, C.S.D., Araújo, R.D.D., Silva, C.E.D., Nascimento, C.C.D., Menezes, V.D.S. and Santos, J.D. 2022. Near-infrared spectroscopy as a tool to discriminate tannins from Amazonian species. *Ciência e Agrotecnologia*, *46*: https://doi.org/10.1590/1413-7054202246001422

Ogawa, S. and Yazaki, Y. 2018. Tannins from *Acacia mearnsii De Wild* Bark: Tannin determination and biological activities. *Molecules*, *23*(4), 837.

Ozaki, Y. 2022. NIR spectroscopy–What a wonderful world! *NIR News*, *33*(1-2), 10-17.

Roux, D.G. 1951. Photometric methods of tannin analysis for Black wattle tannin. *Journal Society of Leather Trades Chemists*, *35*, 322-337.

Roux, D.G. 1957. The Estimation of tannins in Black wattle barks and commercial Mimosa Extracts. *Journal Society of Leather Trades Chemists*, *61*, 275 – 280

Schimleck, L.R. and Yazaki, Y. 2003. Analysis of Black wattle (*Acacia mearnsii De wild*) Bark by Near Infrared Spectroscopy. *Holzforschung*, *57*(5), 527-532.

Siesler, H.W., Kawata, S., Heise, H.M. and Ozaki, Y. (Eds). 2008. *Near-infrared Spectroscopy: Principles, Instruments, Applications*. Wiley – VCH. Germany. Pp 179 – 213.

Slabbert, N.P. 1992. Complexation of condensed tannins with metal ions. plant polyphenols. *Wattle Industry Centre.* 421 – 436.

Tomlinson, G. 2022. Personal communication with Mr. G. Tomlinson. Research and development manager, UCL, Dalton, September 2022.

Williams, P. 2001. Implementation of near-infrared technology. *Near-infrared technology in the agricultural and food industries*.

Williams, P., Manley, M. and Antoniszyn, J. 2019. *Near Infrared Technology: Getting the Best Out of Light*. African Sun Media (e-book).

Yazaki, Y., Gu, R., Lin, Y., Chen, W., and Nguyen, N.K. 1993. Analyses of black wattle (*Acacia mearnsii*) tannins - relationships among the hide-powder, the Stiasny, and the ultra-violet (UV) methods. *Environmental Science* 47, 57-61.

Xiaobo, Z., Jiewen, Z., Povey, M.J., Holmes, M. and Hanpin, M. 2010. Variables selection methods in near-infrared spectroscopy. *Analytica Chimica Acta*, *667*(1-2), 14-32.

Zhu, M.Z., Wen, B., Wu, H., Li, J., Lin, H., Li, Q., Li, Y., Huang, J. and Liu, Z. 2019. The quality control of tea by near-infrared reflectance (NIR) spectroscopy and chemometrics. *Journal of Spectroscopy* 58, 1-11.

Chapter 4: Ageing, climatic, physical, and site effects on black wattle (*Acacia mearnsii* de Wild.) bark

P. Avadianund Bridglall, M.D. Laing, C. Morris, and R.J. Burgdorf

4.1 Abstract

Black wattle (*Acacia mearnsii De Wild*) is a key tree crop in South Africa, valued for its bark and timber, both significant contributors to export revenue. Wattle bark harvesting begins with the rainy season in September and extends to May. During this time, harvested bark is transported to three processing facilities. The journey, which includes stripping and transit lasting several days, exposes the bark to varying environmental conditions, such as temperature, rainfall, humidity, and light, all of which can influence the quality of the bark. The decrease in extractives, darkening of the bark, loss of tannins are all examples of bark quality degradation. To replicate post-harvest conditions, experiments were conducted using fresh bark samples collected bi-monthly from September 2020 to July 2021. These samples were subjected to varying temperature, light, and moisture conditions to simulate real-life scenarios and quantify the extent of bark quality degradation. Quality parameters, including total extractives, tannin content, and Lovibond color (red), were analysed. Advanced statistical techniques, such as principal component analysis (PCA) and redundancy analysis (RDA), were used to identify patterns and relationships among variables. The findings revealed that seasonal changes and site-specific conditions significantly influenced bark quality, particularly affecting Lovibond color, a key quality indicator. This study underscores the impact of pre-extraction environmental conditions on the quality of bark extractives. Developing strategies to mitigate these effects is essential to minimize variability and ensure consistent production of high-quality products. The study also highlights the need for more in-depth and future work.

Keywords: Wattle, Bark, Quality, Tannin, Lovibond color

4.2 Introduction

Various regions of South Africa are climatically suitable for wattle cultivation (Chaunbi, 1997). Although both black wattle (*Acacia mearnsii*) and green wattle (*Acacia decurrens* var. *dealbata* (Link) F. Muell) are cultivated commercially, only black wattle holds significant economic value in South Africa. It is the preferred species due to its dual utility, providing both valuable bark and timber (Dunlop and MacLennan, 2002), the added value of the wattle bark determines the harvesting period, which extends for the duration of the rainy season. In winter, the cold, dry conditions stop the manual stripping of bark (Mackenzie, 1962). The bark is processed at three factories situated in the main production areas, with an average production of 45 000 tons of wattle bark extract powder annually (Chan *et al.,* 2015). Fresh black wattle bark should ideally arrive at the factory as quickly as possible after stripping, *i.e*., on the same afternoon as harvesting (Giannotas *et al*, 2021). This is to reduce water loss and other forms of bark deterioration. Fermentation and oxidation of the bark constituents can set in rapidly, reducing bark quality (Beard, 1957).

Bark quality is assessed based on the percentage of extractives, tannin content, and bark color. Higher levels of extractives and tannins are considered desirable. Bark that is pale in appearance and has low Lovibond color is preferred (Elmer *et al.,*1964). Bark quality is primarily determined by its tannin content because this is the major component of the extractives from the bark. Tannin values may range from 27.1 % to 41.8 %, with a mean value of 36.8 % (on a moisture-free basis) (Sherry, 1971). The levels of tannin can be correlated to several factors, which include the distance of the plantation from the sea, altitude, rainfall per year, mean annual precipitation, and soil-related factors. However, even the most significant site related factor only accounts for 20 % of the variance in total extractives (Gordon-Gray,1965), indicating that there are other factors affecting the bark that have not been documented. However, it is known that superior quality bark in terms of extractives, tannin, and color is produced in late summer or early autumn, and the worst quality is in early spring (Beard, 1957). These influences need to be investigated to understand the drivers of bark quality.

One of the major determinants of the amount of extractives present is the moisture level of the bark. The higher the moisture level, the lower the concentration of extractives in the bark because the mass of the raw material is greater. Moisture levels are affected by the time of delivery to the factory, the age of the tree, and the climate (Nicholson, 1991). The color of the bark extractives is a crucial quality parameter, determining the commercial value of the extractives, where pale extractives are highly valued for leather tanning, and dark extractives have a much lower value. As a result, the color of wattle bark consignments is important in the grading of the bark for quality, and hence the price paid to the farmer for the bark consignments that are delivered to the factories or depots (Kassier, 1959). However, there are no studies in the literature linking environmental factors to the color of the bark and the bark extractives. The farmers are paid for each consignment of wattle bark based on the color and presentation of each consignment of wattle bark, and therefore it is in their best interest to deliver light-colored bark. However, this is largely out of the farmer's control (Phillips, 2020). The stripping of the trees usually takes place in the wet season, and continuously moist conditions cause discoloration of the bark, especially on the inner surface. Moist conditions can also bring about the loss of tannins and non-tannins by fermentation in the consignment, affecting the quality of the final extract product. Elevated temperatures and direct sunlight also accelerate discoloration, leading to poor-quality bark (Dunlop *et al.,* 2003).

Climate (weather) and soil characteristics are two of the major factors that affect tree growth and quality (tannin content and extractives), with one study of black wattle showing that 90 % of the variation between trees was due to soil characteristics (Schönau and Aldworth, 1991). There is limited to no information on the effect on bark color.

This study aimed to investigate the effect of the climate, soil, and exposure to variable environmental conditions after harvesting, on wattle bark quality parameters. The main quality characteristic that was evaluated was the Lovibond color of the bark extractives. The objectives were to determine to what extent the different simulated conditions, as well as the site’s climatic and soil characteristics, affected the color of the bark and the bark extractives.

4.3 Materials and methods

The research was carried out during 2020/2021 at the Institute for Commercial Forestry Research (ICFR). Fresh Black wattle (*Acacia mearnsii De Wild)* bark and soil samples were taken every second month from the Harden Heights plantation (GPS coordinates - 29.2667° S, 30.6167° E) in KwaZulu-Natal. Factory bark samples were also collected during this period for color analysis.

4.3.1 Black wattle bark and soil sample collection, treatment, and analysis

4.3.1.1 Black wattle bark sample collection

Fresh black wattle bark samples were collected every second month, at the start of the rainy season, from September 2020, until July 2021. Four trees were sampled at every sampling event within 24 hours. Bark samples were taken at a DBH of 1.3 m using a stainless-steel cleaver. The band width of the samples was 10 cm, and the sample was taken around the circumference of the tree. Figure 4.1 illustrates (A) the band width of each wattle tree sample collected and (B) the specific section of bark selected for processing.

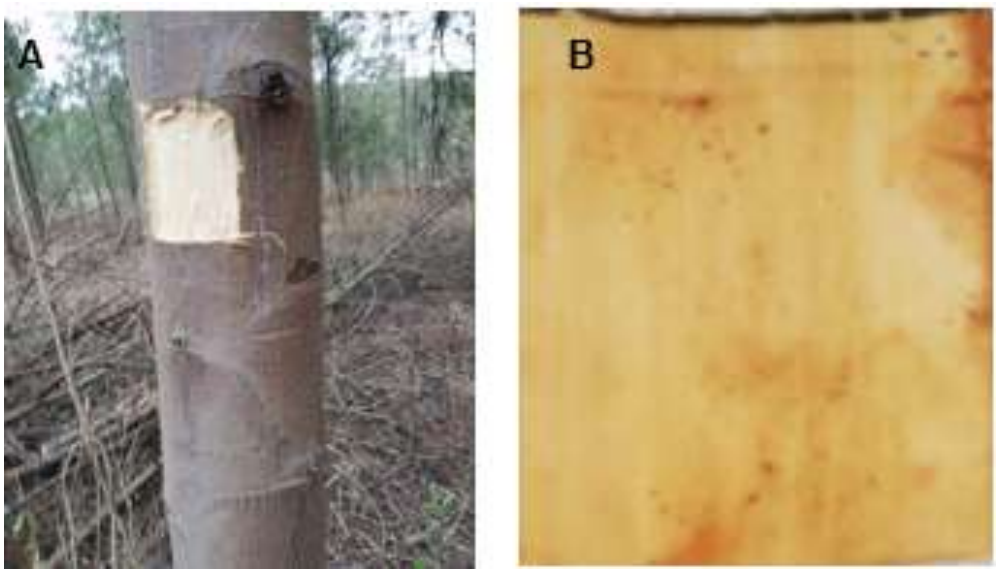


Figure 4.1 An illustration of the bark sampling procedure is shown, highlighting (A) the window section removed from each tree and (B) the piece of fresh bark selected for further processing.

The bark samples were placed in plastic bags, vacuum-sealed, and placed in a cooler box, with ice blocks to prevent degradation. The samples were transported to the ICFR laboratory within 2 hours for further processing.

4.3.1.2 Black wattle bark treatment

Individual tree bark samples were split into 17 samples using a steel guillotine (Dahle, 561 Premium Trimmer). The number of samples per sampling event was n = 64, and a total of n = 340 samples were collected for the entire experiment.

The collected bark samples were exposed to various levels of light, temperature, and moisture at the ICFR laboratory. The treatment combinations were designed to simulate the main environmental effects that harvested wattle bark may be exposed to during handling, transport, and storage. These controlled variations were essential to ensure that the Near Infrared Spectroscopy (NIRS) calibration model incorporated sufficient physical and chemical variability representative of real-world conditions. NIRS scans were acquired for all treated samples, and the resulting spectra were used to develop robust calibration models for predicting bark quality parameters and extractive composition. Incorporating environmentally induced variation is critical in NIRS model development, as spectral responses are influenced not only by chemical composition but also by moisture content, temperature exposure, and structural changes in the bark matrix, as discussed by Workman (2007).

The samples were split into two equal groups that were placed in two identical incubators (Labcon, Low-Temperature Incubator, Model LTIE). One incubator was set at a high temperature, 35 ˚C ± 1 ˚C, and the other at a low temperature, 15 ˚C ± 1 ˚C, to simulate the upper and lower temperature ranges that harvested wattle bark would typically be exposed to. These temperatures are based on upper and lower temperatures within the region (Harden Heights, KwaZulu) (Pers. Comm., Germishuizen,2022).

Samples were taken from fresh untreated bark (t = 0 h), exposed to various light, temperature, and moisture conditions, and sampled again after two days (t= 48 h) and four days (t = 96 h). This was done to simulate the typical time delay between bark harvesting, transport, and processing at a factory, which can take up to four days from harvesting.

Half of the bark samples in each incubator were moistened twice daily, using a mist bottled, for the duration of their incubation to simulate high moisture conditions, caused by rainfall or dew in the field. The other half was not moistened, to simulate dry conditions.

Day and night conditions were also simulated in a 16:8 (day: night) ratio. Artificial light sources were used to simulate daylight in the incubators, as listed below, and were switched on and off automatically at the specified times:

- Philips TL8WY08 F8T5BLB (Japan) as a source of UV light.
- Atelec FLA – 30112 -1 CW 240V Fluorescent tube.
- Full spectrum 600W LED hydro grow light (126X – Pro LED Grow Light) to simulate daylight.

The simulated day (A) and night (B) conditions in the incubators are shown in Figure 4.2.

Incubator A **Incubator B**

Figure 4.2 An incubator set up for the treatment of black wattle bark samples to simulate the ageing and environmental effects that would occur after harvesting. Incubator A simulated daylight, and Incubator B simulating night conditions.

Brown paper bags were used to cover samples that were not exposed to light during day cycles, which simulated conditions of limited light exposure, *i.e*., bark at the bottom of a pile, or within a pile, being stored or transported, to test whether light exposure had any direct or interactive effects on bark extractives content and color. The combination of treatments is outlined in Table 4.1.

Table 4.1 Treatment combinations of black wattle bark samples. The crosses (X) are an indicator of the treatment parameter being tested. Eight different treatment combinations were applied.

Treatment Parameter		Treatment Combination							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Temperature	Low 15 ◦C	X	X	X	X				
	High 35 ◦C					X	X	X	X
Moisture	Low (Not sprayed with water)	X	X			X	X		
	High (Sprayed with water twice daily)			X	X			X	X
Light	Light (Exposed)	X		X		X		X	
	Dark (Stored in a brown paper bag)		X		X		X		X

A list of the various treatment combinations is provided below:

1: Low temperature + low moisture + exposed to light

2: Low temperature + low moisture + not exposed to light

3: Low temperature + high moisture + exposed to light

4: Low temperature + high moisture + not exposed to light

5: High temperature + low moisture + exposed to light

6: High temperature + low moisture + not exposed to light

7: High temperature + high moisture + exposed to light

8: High temperature + high moisture + not exposed to light

The treatment combinations enabled a comprehensive analysis of the effects of environmental factors and their interactions on bark quality.

The fresh and treated bark samples were freeze-dried after the designated treatment using a freeze dryer (Virtis BT Pro series) at -70 ◦C and 175 mTorr for 48 hours, until constant mass was achieved.

The samples were then milled to 0.5 millimetres (mm) using a ZM200 Retsch mill (Retsch, USA). The milled and freeze-dried bark samples were dried until they had a

moisture content of less than 1 %. The samples were then stored at -20 ˚C to prevent sample degradation before analysis.

4.3.1.3 Black wattle bark analysis

The analysis of the bark samples was separated into two groups.

A. Analysis of fresh black wattle bark samples

The milled, freeze-dried, fresh bark samples were extracted using a double autoclave water (DAW) method for bark samples (Avadianund Bridglall *et al.,* 2025). The extractives solution was analysed by the Society of Leather Technologists and Chemists (SLTC) methods for the following parameters: total extractives, tannin, non-tannin, insoluble components, Lovibond Red color, and Lovibond Yellow color.

Quantitative analysis of micronutrients and macronutrients was conducted on milled, freeze-dried bark samples that were ashed in a muffle furnace (Nabertherm, China) and subsequently digested with 16 % hydrochloric acid. Mixed standard solutions were prepared from certified reference standards (De Bruyn Spectroscopic Solutions, South Africa) for Aluminum (Al), Boron (B), Calcium (Ca), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Phosphorus (P), and Zinc (Zn). Samples were analyzed on an Agilent 4100u Microwave Plasma Atomic Emission Spectrometer (MP-AES) (Agilent, USA) against standard reference curves and the results were reported in mg kg^{-1}.

The samples were also analysed for total organic and inorganic Carbon (C), Nitrogen (N), and Sulphur (S) on a Leco Trumac CNS Analyser (Leco, USA).

B. Analysis of treated black wattle bark samples

Twenty percent of the treated bark samples (n = 70) were randomly selected and analysed by the SLTC methods for total extractives, tannin, non-tannin, insoluble components, Lovibond Red color, and Lovibond Yellow color. These samples were used to create a treated bark validation model using a Bruker MPA FT-NIR spectrometer (Bruker, USA). The remaining 80% (n =240) of milled freeze-dried-treated bark samples were scanned on an NIR device (Bruker MPA FT-NIR spectrometer), and the SLTC parameters were predicted using the Quant Analysis module of the OPUS software package (version 8.5, Bruker, Germany). The method for bark scanning on NIRS was carried out was as described in Avadianund Bridglall (2025).

4.3.1.4 Soil sample collection

Soil samples were taken at each bark sampling event, from September to April. Soil samples were taken with a Dutch auger at 0 – 15 cm, and 15 – 30 cm, at the base of each tree that was sampled for bark. The samples were placed in plastic bags and kept cool in a cooler box and transported to the ICFR. The samples were air-dried for 2 - 3 weeks before they were milled and sieved to 2.0 mm and 0.5 mm for analysis at the ICFR. All soil samples were analysed at the ICFR laboratory using the standard ICFR methods for soil analysis, as outlined in the ICFR analytical services (ALS) laboratory manual, adapted from Donkin *et al.* (1993).

4.3.1.5 Soil sample analysis

The three soil samples taken at each site were combined for analysis. The samples were analysed for a list of parameters as specified below:

Moisture factor (mf): The moisture content or mf was calculated by dividing the mass of the air-dried sample (dried for approximately two weeks) by the mass of an oven-dried sample, which was dried overnight at 105 °C for 24 hours in a Drying Oven (Memmert, USA).

pH (potassium chloride KCl): 8.0 g of 2 mm sieved, and air-dried soil was placed in a glass with 20 mL 1M KCl solution. The soil and liquid mixture were left to equilibrate overnight at room temperature. The supernatant pH was measured using a pH probe on an Orion Star A200 pH meter (Thermo Fisher Scientific, USA) calibrated with pH 7.0 and pH 4.0 calibration solutions (Merck, Germany).

pH (water H_2O): 8.0 g of 2 mm sieved air-dried soil sample was placed in a glass tube and 20 mL of deionized water was added. This mixture was stirred thoroughly and left to stand overnight at room temperature. The supernatant pH was measured using a pH probe on an Orion Star A200 pH meter (Thermo Fisher Scientific, USA) calibrated with pH 7.00 and pH 4.00 calibration solutions (Merck (Germany).

Exchangeable Acidity: this was determined using a titration method whereby a 1M KCl (Merck, Germany) solution was added to the soil sample and water. A few drops of phenolphthalein indicator solution (1 g of phenolphthalein (Merck, Germany) in 50 mL of 100 % ethanol and 50 mL deionized water) is added and the samples are titrated using a 0.01 M Sodium Hydroxide (NaOH) (Merck, Germany) solution to the

endpoint, when a pink color is reached and maintained for at least 30 seconds. The exchangeable acidity can then be calculated from the equation:

(mL NaOH x c. NaOH x mf x 100 x volume. extract) ÷ (volume. aliquot x mass of soil used)

The results were reported in $cmol_c\ kg^{-1}$ soil.

Exchangeable Cations: 25 mL 1M ammonium acetate $C_2H_7NO_2$ (Merck, Germany) (adjusted to pH 7.0 with acetic acid (CH_3COOH) or NaOH as necessary) was added to 5.0 g air-dried, 2 mm sieved soil and shaken on a reciprocating shaker (Model no: SPLMP8TUPF55 – Lab-Design Engineering, Labcon, South Africa) for 10 min at 170 revolutions per minute (rpm), and thereafter centrifuged (Centrifuge Uniscen, Orto Alresa, Spain) at 3000 rpm for 2 min. The supernatant was filtered through Whatman no. 42 filter paper (Merck, Germany). The filtered sample was then analyzed on an Agilent 4100 Microwave Plasma Atomic Emission Spectrometer (MP-AES) (Agilent, USA). The spectral lines and calibration standard concentrations were as per Table 4.2 below, which were combined in 1M pH 7.0 $C_2H_7NO_2$ extraction solution, at each respective concentration.

Table 4.2 Spectral wavelength and calibration standard solutions for exchangeable cations measured on the Agilent 4100 Microwave Plasma Atomic Emission Spectrometer (MP-AES).

Element	Spectral wavelength (nm)	Standards solutions concentrations (mg kg^{-1})
Calcium	430.253	0, 100, 200, 300, 600
Magnesium	383.829	0,5,10, 15, 30
Potassium	769.897	0, 5, 10, 15, 30
Sodium	588.995	0, 0.5, 1, 2, 4

Results were converted to oven-dried mass and were reported in centimol positive charge per kg of soil ($cmolc\ kg^{-1}$).

Soil Macronutrients: The soil macronutrients Cu, Fe, Mn, and Zn were quantitatively determined using an Agilent 4100 Microwave Plasma Atomic Emission

Spectrometer (MP-AES) (Agilent, USA) against standard reference curves and the results were reported in mg kg^{-1}.

Bray II Phosphorous: This was performed as per Bray and Kurtz (1945) to determine the total, organic, and available forms of phosphorus in the soil samples.

Total % Carbon, Nitrogen and Sulphur (CNS): 0.5mm sieved, and air-dried soil samples were analyzed on a Leco Trumac CNS analyzer (Leco, USA). The C, N and S levels were plotted graphically using Microsoft Excel.

4.3.2 Factory bark sample collection and analysis

Black wattle bark samples were collected individually at the three wattle extract factories in South Africa from November 2020 to May 2021. Due to the confidentiality requirement of the factories, the factories were identified as A, B, and C for this study. The samples were collected only when the factories were operational and were producing wattle bark extracts. These were from harvested bark consignments that had been delivered to the factories for the manufacture of wattle extract powders and solid extract. Bulk grab samples were collected by the factory; these samples were taken off the conveyer belt after the bulk was chopped/ chipped. The conveyer belt takes the samples to the pots for extractions at the factory . The samples were stored in a freezer at - 18 ˚C, transported to the ICFR under cold-chain conditions, freeze-dried immediately and milled, once dry. The milled, freeze-dried bark samples were stored in a freezer (- 20 ˚C) to prevent sample degradation before further analysis.

A portion (50 %) of the factory-milled, freeze-dried, bark samples were first scanned on the Bruker MPA NIR device and then analysed by the SLTC methods for extractives, tannin, non-tannin, insoluble, Lovibond Red color, and Lovibond Yellow color. Micronutrients, macronutrients, and CNS were also determined. The wet chemistry analyses provided the reference chemistry used to create a factory bark NIRS calibration model. The remaining 50 % of milled, freeze-dried bark samples were also scanned on the NIRS, and the SLTC parameters were predicted using the predictive model produced in the Quant Analysis module of the OPUS software package (version 8.5, Bruker, Germany).

4.3.3 Weather variables of Harden Heights

The mean minimum temperature (TMN), maximum temperature (TMX), rainfall, relative humidity (RHP), solar radiation (SRAD), and evapotranspiration (EVP) were recorded from five sites near Harden Heights using the forestry company's cadastral boundaries, and the location of the weather stations (SASRI weather web and South African Weather Services (Pers. Comm., Germishuizen,2022), as shown in Figure 4.3. The readings are captured on a monthly basis, and the average was used in this study.

Figure 4.3 Map of weather stations near Harden Heights plantations (GPS coordinates 29.2667° S, 30.6167° E) in Kwa-Zulu-Natal (Source: the ICFR Weather database and SASRI Weather Web (Germishuizen)).

4.3.4 Statistical Analysis

The measured parameters of all bark samples were compared statistically using Microsoft Excel (Version 2207 Build 16.0.15427.20166) and R (CRAN, version

4.2.0). Cinco (Version 5.12) was used for principal component analysis (PCA) and redundancy analysis (RDA). Permutation multivariate analysis of variance (PERMANOVA) was performed using Primer (Version 6), and univariate analyses ANOVA was done in Past 3 software (Version 3.20). The PCA and RDA are both multivariate techniques used for analysing relationships between variables (Zuur *et al.,* 2007), which were used in this research to identify patterns in the data. Closely correlated variables are found in the same gradient with their arrows pointing in the same direction. Univariate ANOVA is a type of ANOVA with only one dependent variable, which works by determining the total variability of the dependent variable between groups and within groups (Haase and Ellis, 1987). A PERMANOVA is a statistical method used to determine the significance of differences between groups of multivariate data (Anderson, 2014).

4.4 Results

4.4.1 The effect of weather, soil, and harvesting practices on black wattle bark

This aspect of the study was performed to understand the site characteristics and their effect on bark quality parameters.

4.4.1.1 Statistical analysis of fresh black wattle bark

The fresh, milled, freeze–dried bark samples were analysed by the SLTC methods, and the bark quality parameters are shown in Figure 4.4. The data showed that there were variations in the bark properties and quality parameters over the sampling duration. Bark moisture increased from the beginning of spring to the peak of the rainy season in January and then declined to lower levels by July. The mean extractive content did not appear to vary considerably during the year. However, mean tannin content appeared to decline gradually from September to March, increased briefly in May, and then decreased substantially in July. Mean non-tannin content increased sharply from September to March and then dropped in May increased slightly in July. This appears to be the inverse of the trend for mean tannin content. Lovibond red and yellow colors showed similar trends to each another, increasing slightly from September to November, then decreasing for the remainder of the rainy season, after which they increase sharply from March to July, which represents the period from the beginning, through to the middle of the dry season

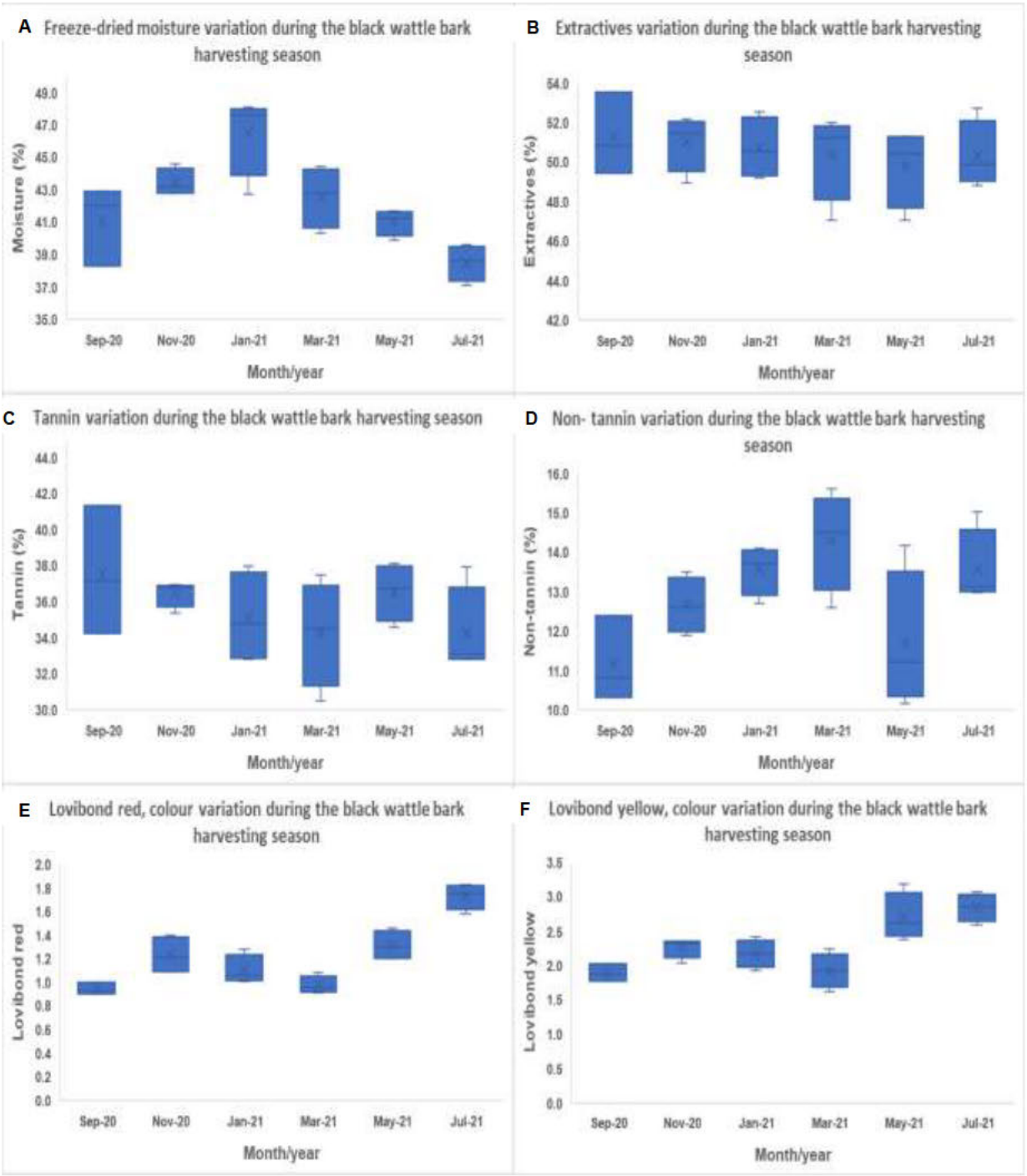


Figure 4.4 Box and whisker plots of the seasonal variation of (A) moisture %, (B) extractives %, (C) tannin %, (D) non-tannin %, (E) Lovibond Red color, and (F) Lovibond Yellow color of fresh, milled, freeze-dried black wattle bark.

The PCA biplot in Figure 4.5 illustrates the relationship between the bark quality parameters, excluding color, and the samples taken during the season. The bark quality parameters were not strongly affected by the sampling month because there are no distinctive clusters. The plot shows that non-tannin % and insoluble

components % had a weak inverse correlation with extractives, tannin, and polyphenolics content, *i.e.,* as the tannin % increases, the non-tannin % will decrease slightly.

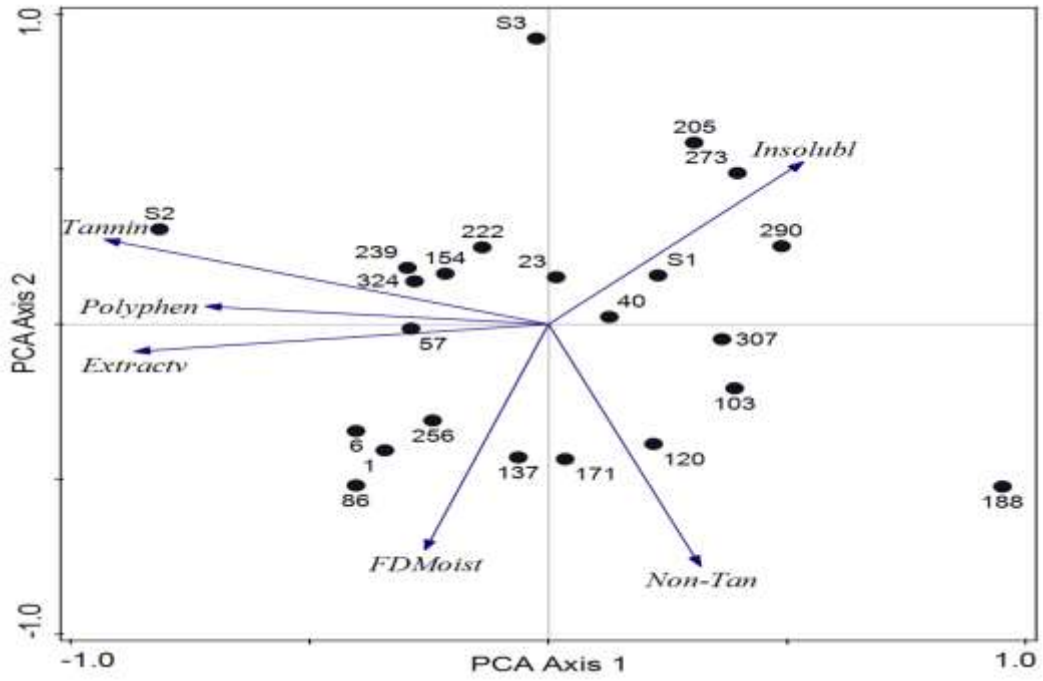


Figure 4.5 A PCA biplot of the bark quality parameters (freeze-dried moisture %, extractives %, tannin %, polyphenolics %, non-tannin %, and insoluble %) in relation to samples obtained over the season. The dots represent the samples collected at intervals over the year.

The PCA biplot in Figure 4.6 plots the Lovibond color values over time, in conjunction with the climatic data. This showed that most of the variation was explained by the first principal component, which was strongly influenced by the weather values and the sampling time and showed distinct clusters. Weather parameters correlated strongly to the rainy season, during which the best extractives colors occurred.

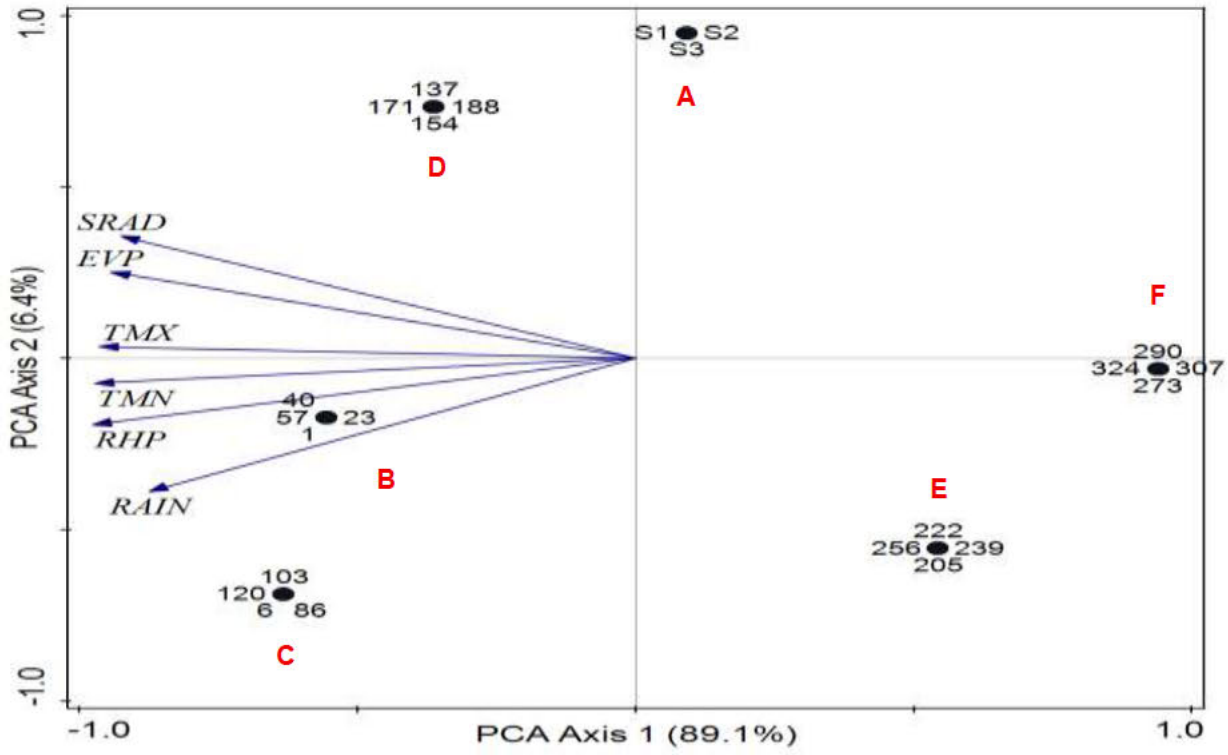


Figure 4.6 A PCA biplot of weather variables (solar radiation (SRAD), evapotranspiration (EVP), maximum temperature (TMX), minimum temperature (TMN), relative humidity RHP, rainfall) at Harden Heights recorded between September 2020 to July 2021. The dots indicate the Lovibond color samples. Sampling months are denoted by the letters: A (September 2020), B (November 2020), C (January 2021), D (March 2021), E (May 2021), and F (July 2021).

The RDA biplot in Figure 4.7. shows that solar radiation (SRAD) was the major factor that strongly and inversely correlated with the Lovibond colors. The effect of SRAD on color was greatest during the colder months, *i.e.,* May, and July (Cluster B), which was strongly associated with an increase in Lovibond Red and Yellow colors, as opposed to samples taken at the start of spring, *i.e.,* in September (Cluster A).

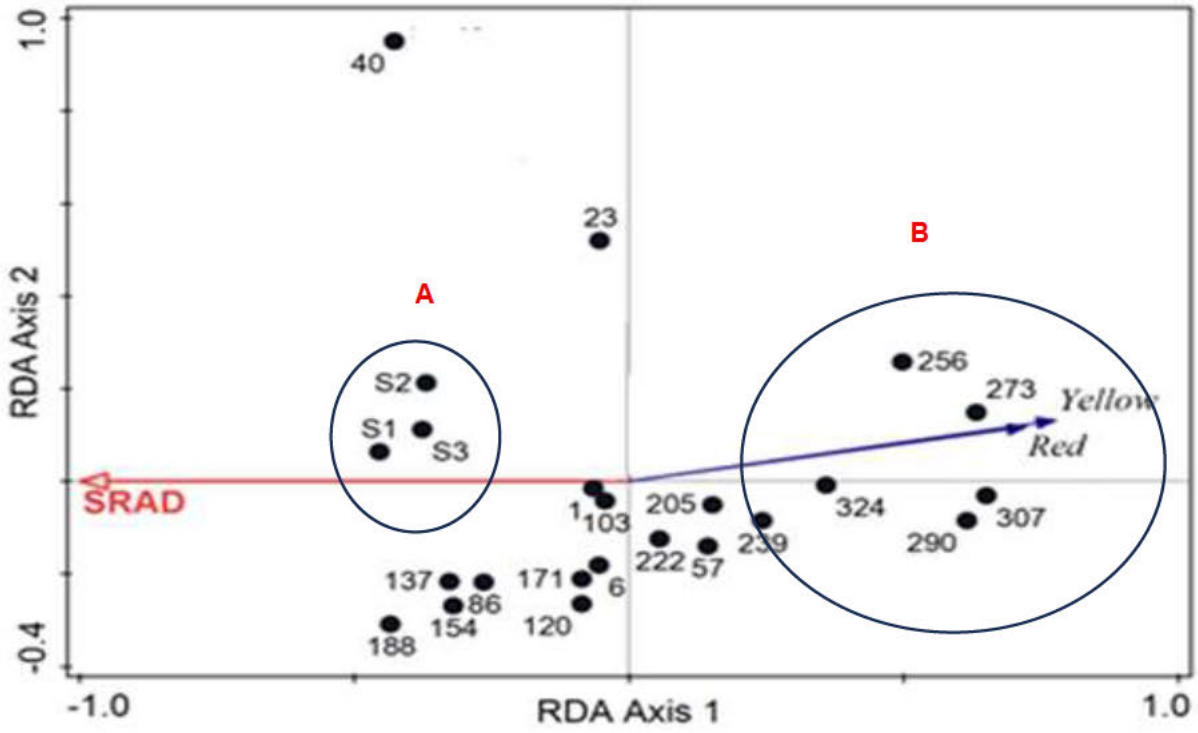


Figure 4.7 RDA biplot showing solar radiation (SRAD) as the largest contributor to lower Lovibond color values. The dots indicate the samples taken at intervals over the year Cluster A represents samples from September 2020 and Cluster B is samples harvested from May 2021 to July 2021.

The PCA biplot in Figure 4.8 plots the fresh bark micro- and macro-nutrients. This indicated that there were no obvious relationships between most of the nutrients, in relation to the sample data collected over the season. However, it was observed that phosphorus (P) and boron (B) had large inverse influences on the samples taken in early winter (Cluster A - July 2021).

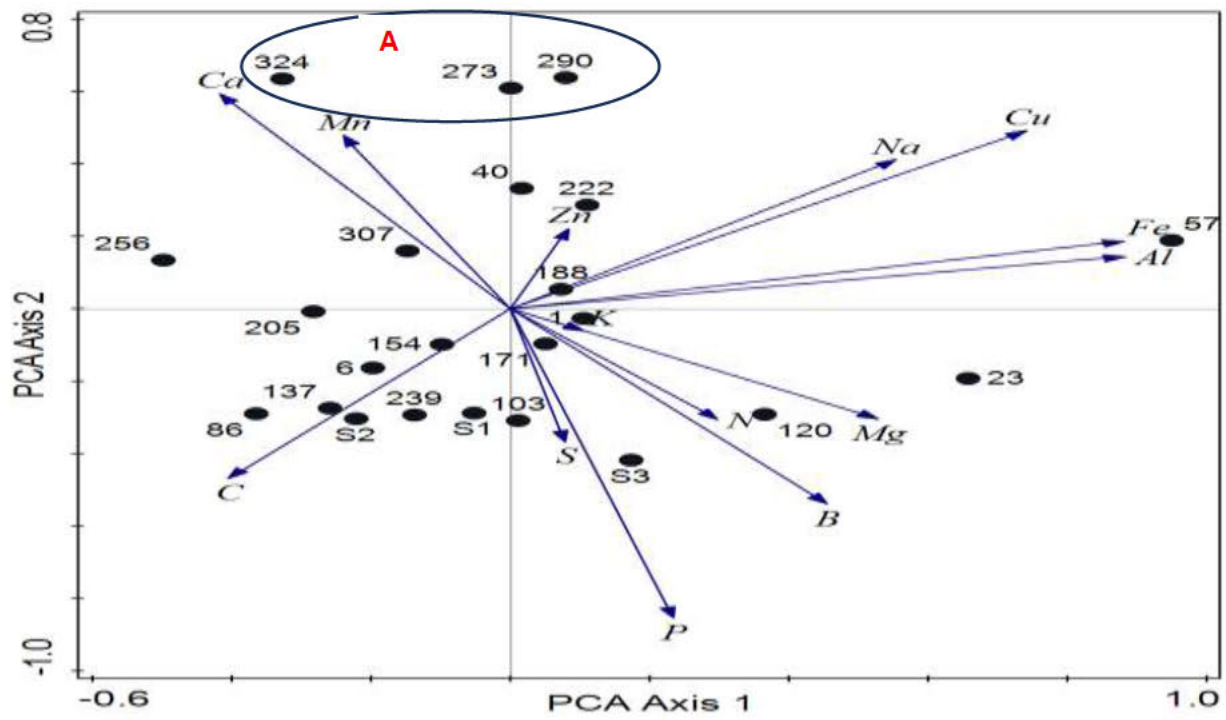


Figure 4.8 PCA biplot of micro- and macro-nutrients of fresh, milled, and freeze-dried bark samples. The dots indicate the sample number, and Cluster A highlights the samples harvested in July 2021.

The RDA biplot in Figure 4.9 shows the major Lovibond color responses to the bark nutrients. Elemental P and N related the most to color variation. Phosphorous appears to have a strong inverse relationship with color, and while nitrogen values varied considerably, this did not strongly influence color.

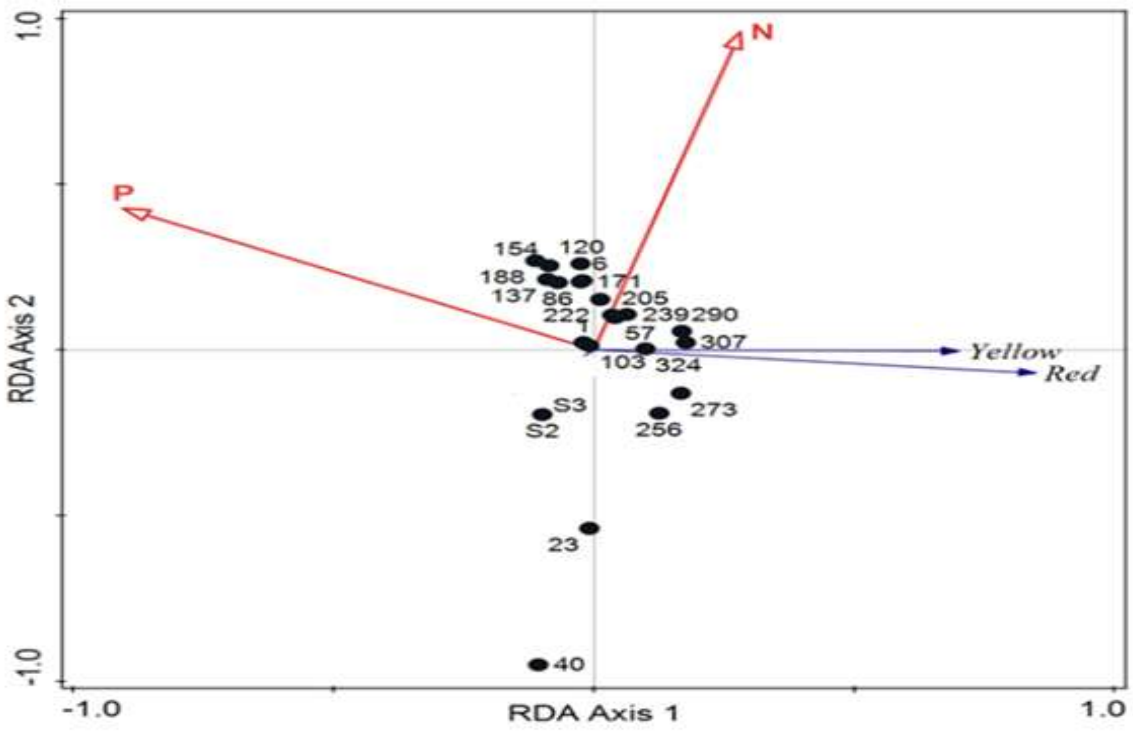


Figure 4.9 The RDA biplot of the relationship between Lovibond color (red and yellow), and phosphorus (P) and nitrogen (N) levels in fresh bark. The dots represent the fresh bark samples taken at intervals over the year.

The soil at Harden Heights was characterized as being composed of 39 % silt, 30 % clay, and 31 % sand. The bar graphs in Figure 4.10 illustrate the analysis of the various soil parameters (A) pH, (B) exchangeable acidity, (C) exchangeable cations (D), macronutrients, (E) Carbon, Nitrogen, and Sulphur (CNS) of the soil, and (F) Phosphorus (P)). Most soil parameters remained consistent over the sampling duration, although some outliers did occur.

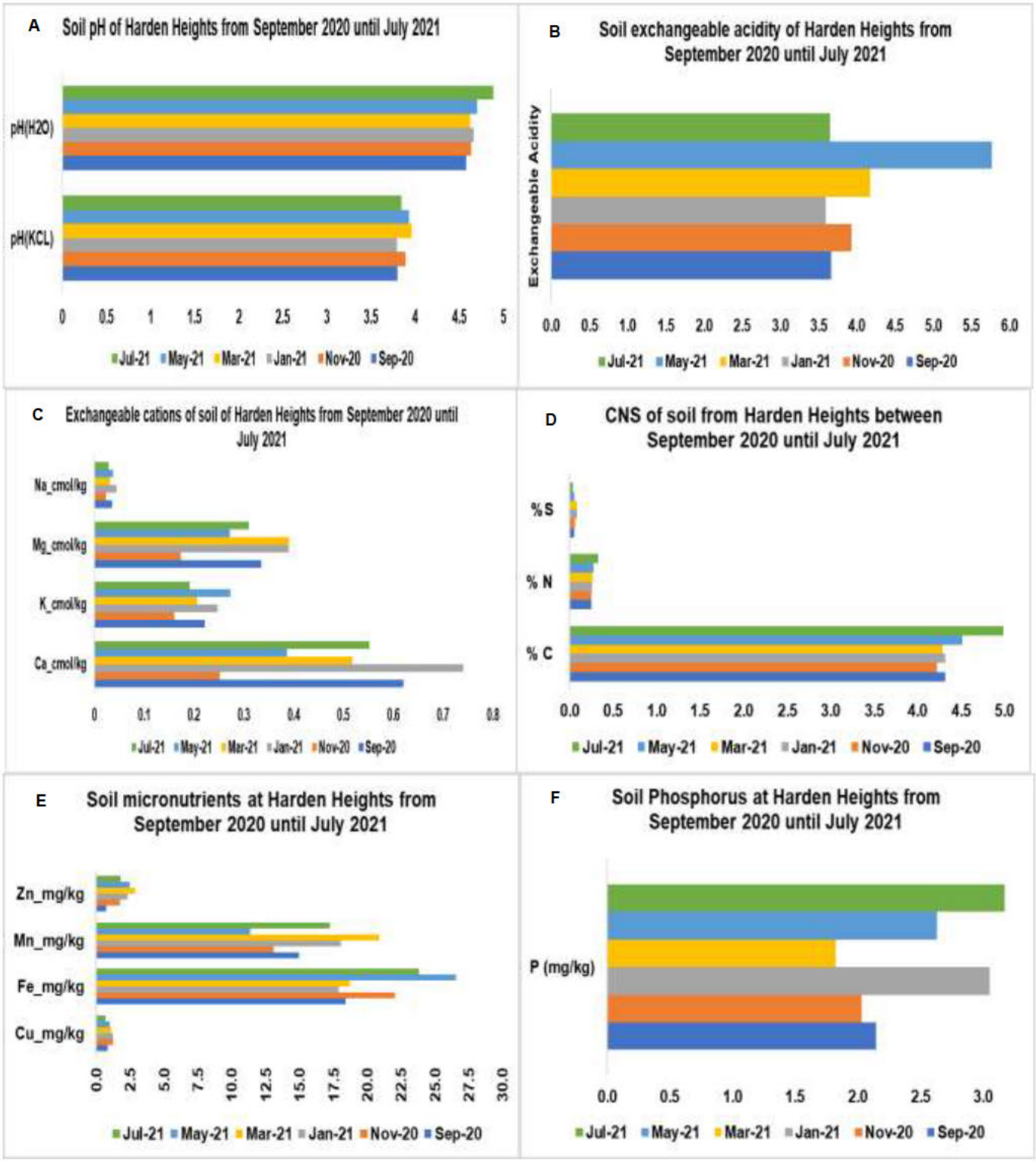


Figure 4.10 Bar graphs for soil variables for soil samples taken at Harden Heights: (A) pH, (B) exchangeable acidity, (C) exchangeable cations, (D) carbon, nitrogen, sulphur (E) soil micronutrients and (F) phosphorous.

The RDA plot in Figure 4.11 shows that the variation in the soil, where sand and iron (Fe) content corresponded most strongly with the bark extractives' Lovibond color variables, while sulphur levels had a strong inverse relationship with color.

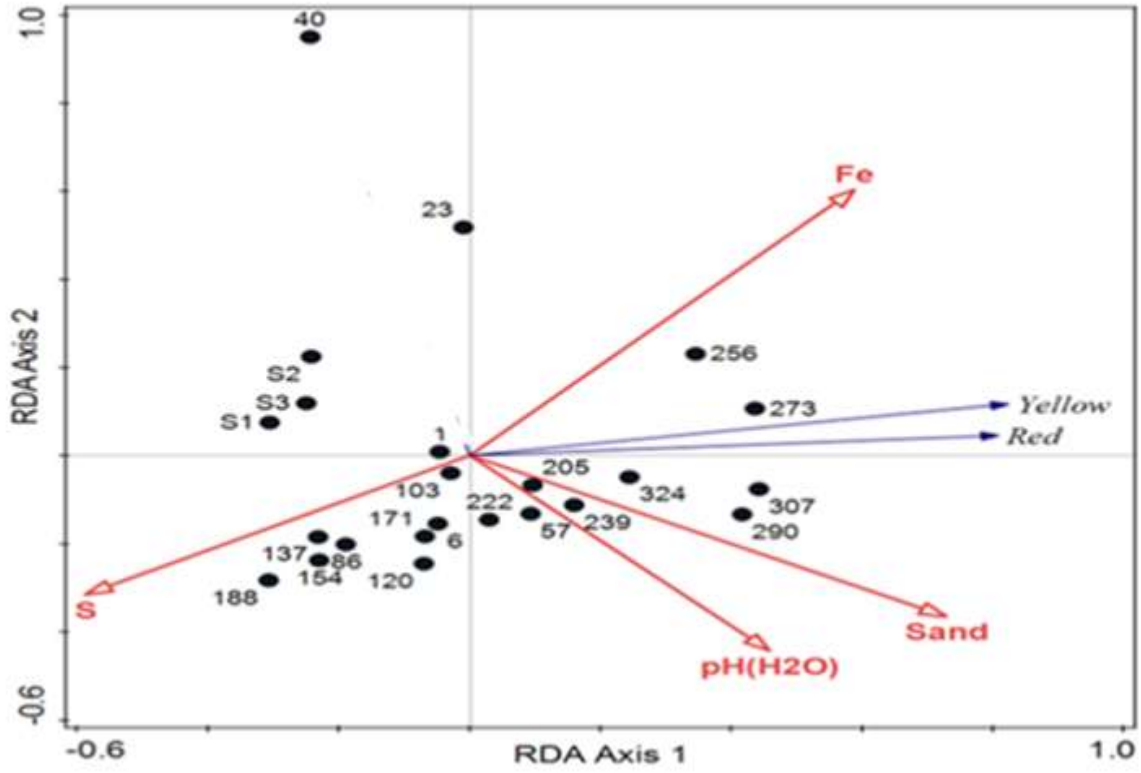


Figure 4.11 The RDA plot of soil properties in relation to bark extractives color. The dots represent the bark samples collected over the sampling period.

The RDA data for the contribution of the various soil factors are summarized in Table 4.3. The data shows the significant effects that sandy soil composition, Fe, pH, and S had on the bark extractives color.

Table 4.3 RDA table showing the contribution of soil variables to Lovibond color (red and yellow) of fresh bark samples.

Soil variable	Explains (%)	Contribution (%)	Pseudo-F	P
Sand	26.6	28.1	7.6	0.003
Fe	17.2	18.1	6.1	0.007
pH (H_2O)	12.8	13.5	5.6	0.006
S	8.4	8.9	4.3	0.014

4.4.2 Statistical analysis of treated bark samples

Figure 4.12 shows an increasing trend in color development (Lovibond Red) over time. The variability of the bark color also increased with time.

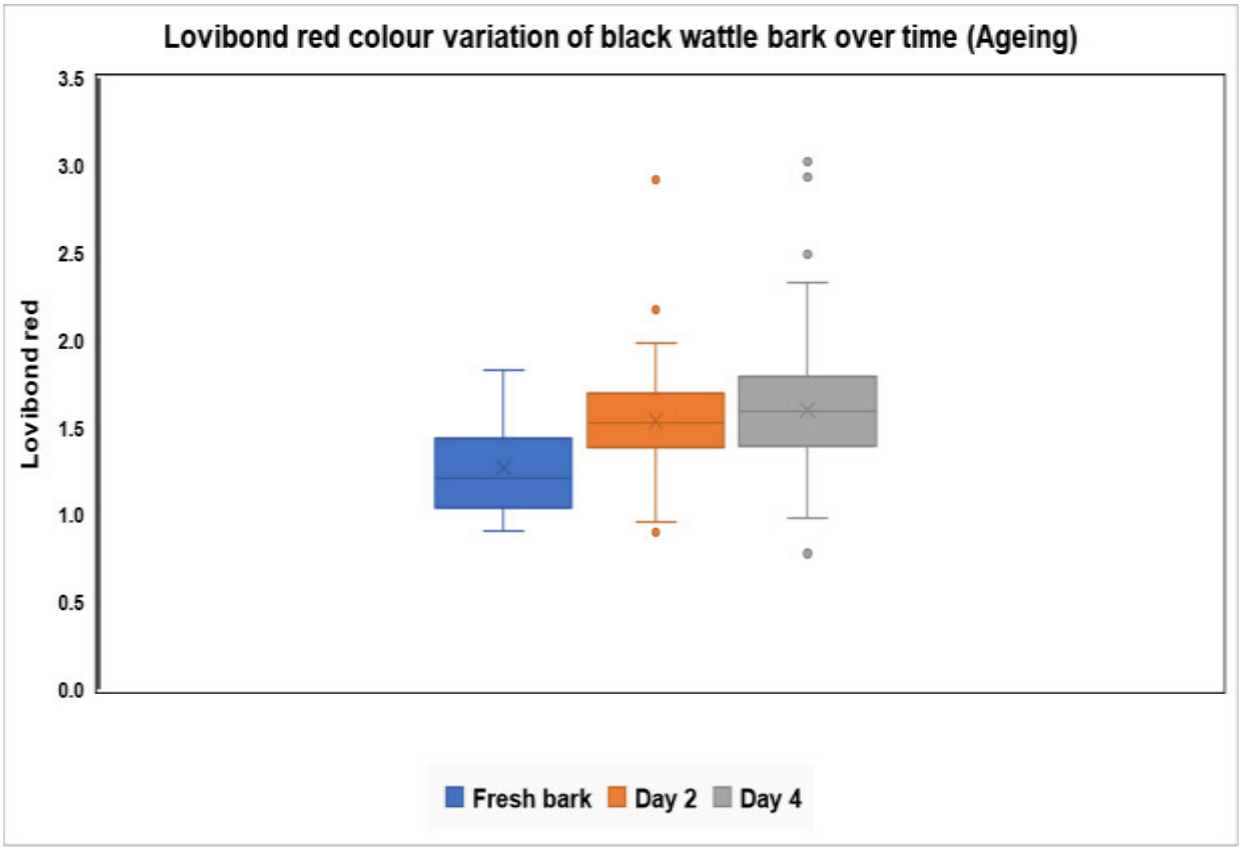


Figure 4.12 A box and whisker plot was used to compare Lovibond Red colors of fresh, 2-day-old, and 4-day-old bark. A lower score for Lovibond Red is better. The whiskers in the plot represent variation from the mean, and the • indicates outliers.

The RDA plot in Figure 4.13 shows the response of extractives %, tannin %, color (Lovibond Red, and Lovibond Yellow) in response to the different treatments (Table 4.1). The control (fresh, untreated bark) was distinct from the treated samples. The analysis indicated increases in tannin content and color (red and yellow) were positively influenced by the higher temperature and higher moisture treatments.

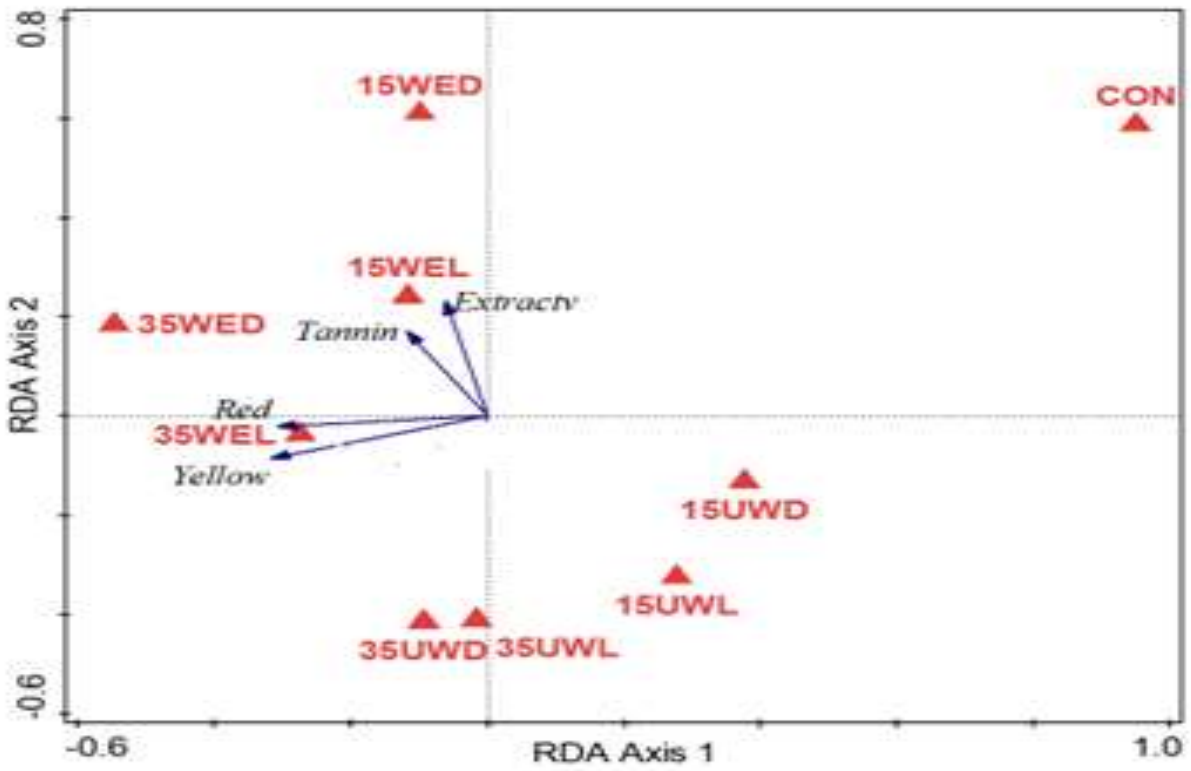


Figure 4.13 The RDA plot of treatments centroids and response variables for different combinations of temperature, moisture, and light for fresh (Control (Con)) and treated bark samples (15 ˚C – wetted - dark (15WED), 15 ˚C – wetted - light (15WEL), 35 ˚C – wetted - dark (35WED), 35 ˚C – wetted - light (35WEL), 15 ˚C – unwetted - dark (15UWD), 15 ˚C – unwetted - light (15UWL), 35˚C – unwetted - dark (35UWD), and 35 ˚C – unwetted - light (35UWL)).

The PERMANOVA results shown in Table 4.4 of temperature, moisture, and light identified the main effects on extractives %, tannin %, and Lovibond color (red and yellow). The main effects of temperature and moisture on tannin % were significant, with no direct effects from light or significant higher-order interactions. The interactions between temperature and moisture, temperature and light, moisture and light, and temperature, moisture, and light were not significant ($p > 0.05$)

Table 4.4 PERMANOVA showing the main and interactive effects of treatments on bark extractives properties.

	df	SS	MS	Pseudo-F	P(perm)	perms
Temperature	1	23.6	23.6	4.9	<0.001	9948
Moisture	1	42.3	42.3	8.7	<0.001	9951
Light	1	5.7	5.7	1.2	0.30	9946
Temperature + moisture	1	3.4	3.4	0.7	0.58	9933
Temperature + light	1	2.9	2.9	0.6	0.66	9950
Moisture + light	1	2.0	2.0	0.4	0.80	9950
Temperature +moisture +light	1	0.5	0.5	0.1	0.99	9940

The RDA plot in Figure 4.14 shows that temperature had the greatest direct influence on the color of the treated bark samples.

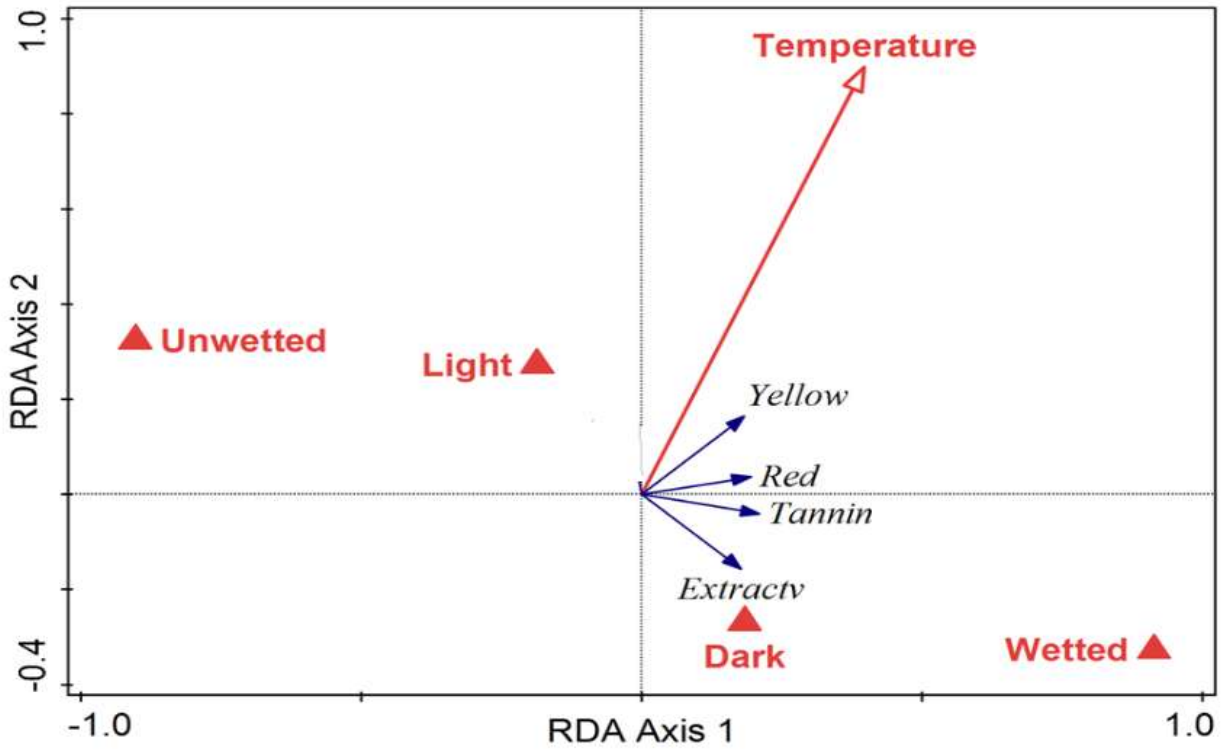


Figure 4.14 RDA plot of the various treatments for bark quality parameters.

The ANOVA in Table 4.5 revealed the direct effects of temperature and moisture. There were significant direct effects from increased moisture on all parameters.

However, temperature only had a direct effect on the Lovibond colors (red and yellow).

Table 4.5 p- values from a two-way ANOVA of temperature and moisture on bark quality parameters.

Treatment variable	Extractives	Tannin	Lovibond Red	Lovibond Yellow
Temperature	0.23	0.45	0.01	<0.001
Moisture	<0.001	<0.001	0.01	0.02

Interactive plots of temperature and moisture in Figure 4.15 show that there were significant interaction effects between temperature and moisture affecting all the measured parameters. Increased moisture increased extractive content at lower temperatures (A). Drier samples had lower tannin levels at lower temperatures (B), while colors always increased with higher moisture at higher temperatures (C and D).

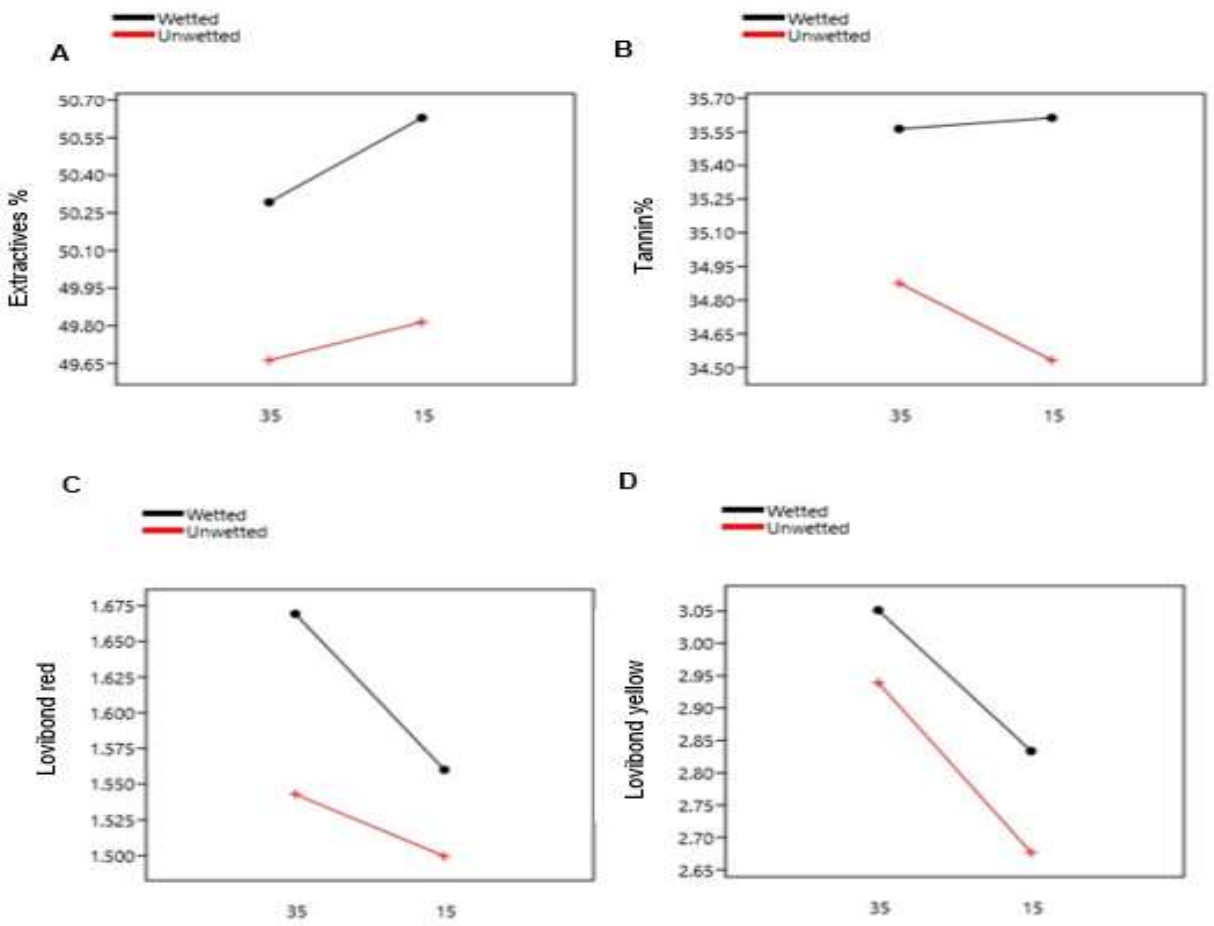


Figure 4.15 Interactive line plot of the temperature x moisture interaction on bark quality parameters (A) extractives %, (B) tannin %, (C) Lovibond Red, (D) Lovibond Yellow.

4.4.3 Analysis of factory bark samples

The plots in Figure 4.16 (A – D) show the variation in the major bark quality parameters (extractives %, tannin %, Lovibond Red, and Lovibond Yellow) of combined factory bark samples harvested during the 2020/2021 season. The extractives range was between 44 % to 58 %, the tannin range was 32 % to 37 %, the Lovibond Red range was 0.8 to 2.2, and the Lovibond Yellow range was between 1.4 to 4.4.

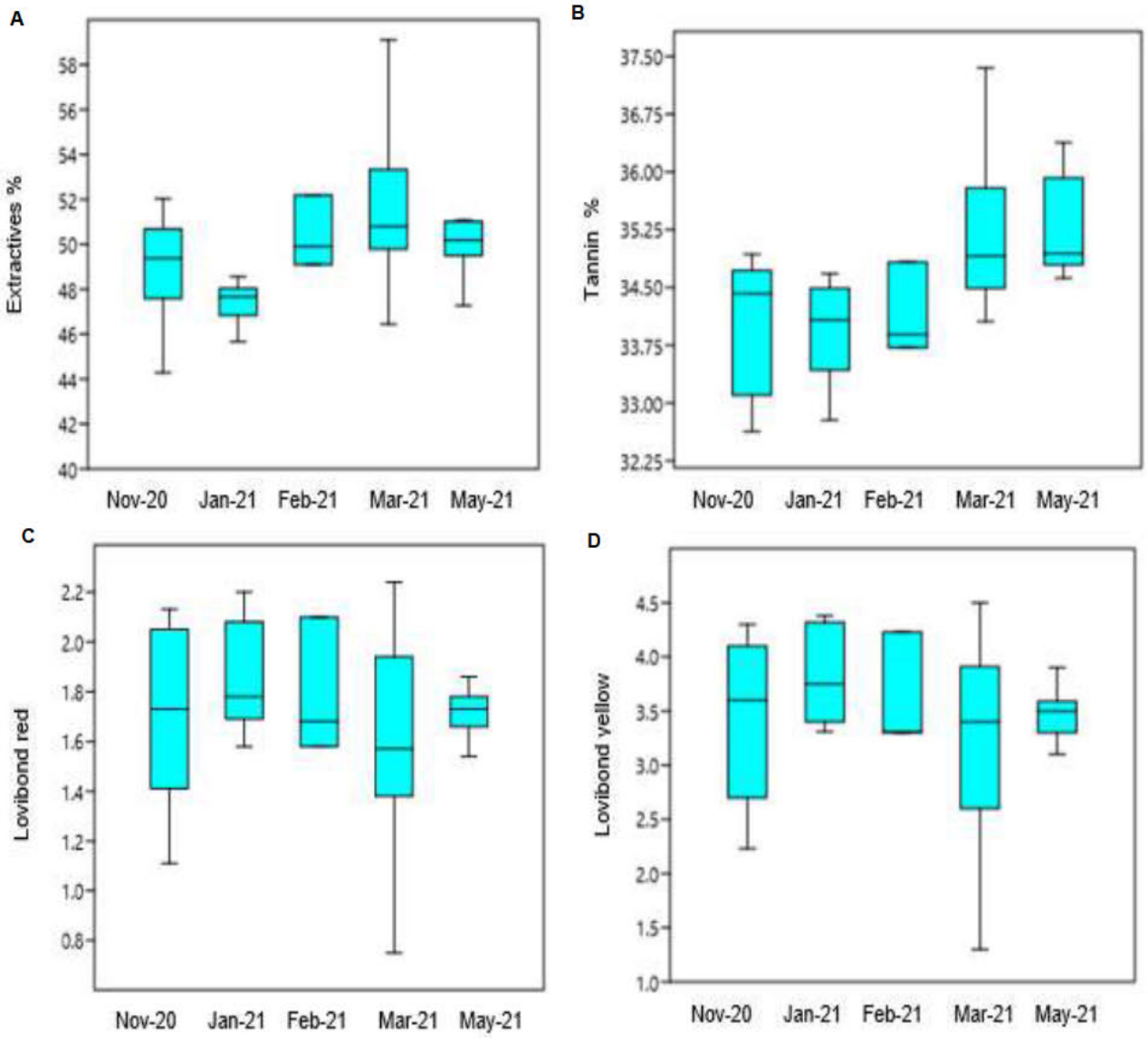


Figure 4.16 Box and whisker plots of the seasonal variation for bark quality parameters (A) extractives %, (B) tannin %, (C) Lovibond Red, and (D) Lovibond Yellow from November 2020 until May 2021.

An RDA plot in Figure 4.17 shows the variables for bark properties of samples sourced from the three wattle bark extraction factories (denoted as A, B, and C), which are in different geographic regions. The plot shows that higher colors, as well as tannin content, were associated with Factories B and C, and most of the other bark parameters were more positively associated with Factory A.

D

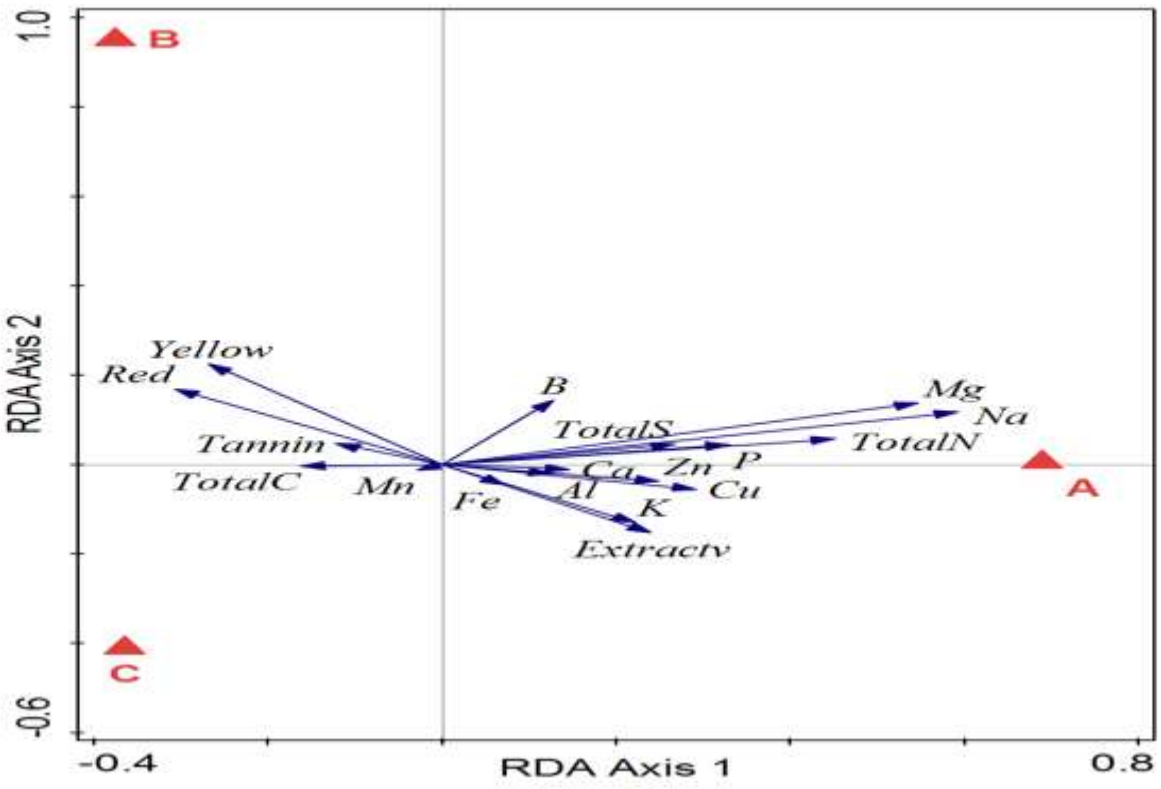


Figure 4.17 RDA plot of the factory (three wattle bark extractives factories in South Africa) bark quality parameters (extractives %, tannin %, Lovibond Red, and Lovibond Yellow) and bark mineral content (micronutrients (Ca, Mg, Na, and K), macronutrients (Al, Fe, Mn, Cu, Zn, and B) and total CNS).

4.5 Discussion

The analysis and exploration of the data obtained from the soils and bark at the sampling site revealed that the agroecological aspects of the plantation site (*i.e.*, soil and climate), and temporal variation impacted on bark extractives quality in terms of color and tannin content, which were the primary parameters of interest in this study. This is significant because it showed that no single factor was responsible for variable bark quality, but that several factors made contributions, which can now be addressed by the development of a range of mitigation strategies, such as a range of fertiliser inputs, as discussed by Titshall (2021).

Climatic and site conditions are considered to be major contributors to the fluctuation in bark quality parameters (Elmer *et al.,*1964). However, seasonal variation investigated in this study was a significant factor affecting bark quality, which may not be of major importance to factories, because the harvest period is out of their

control. Black wattle bark is stripped during the six to eight months of the year that have adequate rainfall, which is the only period when the bark is of suitable strippable quality for subsequent processing at the extractives factories (Hu *et al.,* 2017). While the bark quality parameters (moisture %, extractives %, tannins %, non-tannins %, and Lovibond color (red and yellow)) fluctuated during the season (Figure 4.3), there were no meaningful trends. However, Figure 4.4 shows that the extractives %, tannin %, and polyphenolic % were strongly inversely correlated to non-tannin%. This suggests that the tannins that are produced during the growth of the tree are not involved in any dynamic processes, *i.e.*, they are not changed or modified during the trees' growth, since they are primarily plant defence chemicals that are stored in tannosomes (Sieniawska and Baj, 2017), while non - tannins, such as sugars and gums, do change structure, quantity, and location, due to seasonal changes, as described by typical tree physiology. The extractives were least affected by seasonal variation, even though the moisture fluctuated during the season, and the relationship between the two is inverse. However, according to Nicholson (1991), the variation in extractives is minimal even under the most adverse conditions, therefore suggesting that it is the tannins and non-tannins levels that vary as the extractive content remains static.

The climatic conditions (SRAD, EVP, TMX, TMN, RHP, and rainfall, Figure 4.5) strongly affected bark quality parameters because tree physiology controls chemical responses to seasonal conditions (Prentice *et al.,* 1992). The SRAD, which is a function of seasonal variation, had the greatest impact on the red and yellow color properties in the colder months (May and July), in comparison to September because the relative tannin content is higher in the colder months (Sherry, 1971), due the proportional relationship between tannins and non-tannins. The wattle tree also begins to flower before the end of the dry season (Milton, 1987) and these physiological changes could also affect color changes just before the harvesting season begins. In the sugar industry, ripening agents are applied just before flowering to inhibit the flowering process, resulting in increased sugar yield (Moore and Berding, 2013). A similar experiment could be proposed for bark extractives, where a chemical treatment is applied to prevent flowering. By inhibiting flowering, this approach could enhance tannin concentration in the bark, potentially improving both the quality and yield of bark extractives.

Currently, the beginning of the harvesting and bark extractives production season is determined by a minimal required rainfall level (Havemann, 1992). However, a better understanding of the seasonal factors could help to optimise harvesting schedules to ensure that the highest quality bark is harvested, and to maximize the harvesting period. In addition, Climate Change is likely to affect bark quality parameters, and the monitoring of the Lovibond Red color in response to global warming across different sites could allow factories to exploit trends towards lighter bark extractives colors. During these periods, they could manufacture premium quality bark extractives products that are light in color, which have a higher market value, and this would mitigate some of the negative financial impacts of Climate Change.

Nutritional factors assessed by the analysis of soil and bark over the harvesting period could not connect changes in bark extractives quality to changes in soil characteristics, largely because the samples were taken from a single site. The results (Figure 4.7) showed relatively indeterminate effects on bark nutrients, and multiple sites need to be studied to identify any relationship between mineral levels and bark quality. However, there was a very strong correlation between bark quality parameters in response to phosphorous levels in the bark. In particular, low levels of P (Figure 4.8) had a significant relationship to darker coloring of the bark extractives. This could be explained by the complexing of phosphorous with iron and aluminium (Hemwall, 1957). However, the low P in the bark could also be specific to the Harden Heights plantation, so additional sites need to be investigated to confirm this effect. If low phosphorus levels are a general problem at multiple sites, then the addition of a P fertiliser could mitigate bark color issues. It is already known that the applications of P fertiliser increase black wattle bark and timber yields (Titshall, 2021). Nitrogen levels were variable and had little impact on color because wattle trees are legumes, and deficiencies in N are remedied by symbiotic microbial activity (Barnet *et al.,* 1985).

The average mineral tests of the plantation at Harden Heights (Figure 4.9) showed little variation during the season. The slight variation that was noted was for soil exchangeable acidity, Ca, soil P, and Fe, which could also be attributed to sampling error or analytical error. However, the RDA plot (Figure 4.10) showed that variation in sand and iron levels had the greatest effect on the bark color variation. Soils in this area have an abundant level of Fe (Herselman, 2007). This is an important

consideration because wattle bark tannins react with iron to form an iron-tannate complex, which is readily oxidised (Hillis, 1997). This results in the darkening of the tannin molecule (Hillis,1997), leading to poor color quality. The production of the iron–tannate complex can be inhibited by using ethylenediaminetetraacetic acid (EDTA), by a mechanism known as the Fenton reaction (Engelmann *et al.,* 2003). The mitigating effect of EDTA was demonstrated by the research conducted by Avadianund Bridglall *et al.,* 2025. Future research could explore the use of safe chelating agents during the extraction process, or the addition of soluble phosphate salts during in the extraction process, which should inhibit the darkening of bark extractives, increasing the quality and value of the bark extractives products.

Aside from genetics and extraction processes, the environmental conditions during harvesting, temporary storage and transport to the extraction factory also affected bark color and tannin content. Hagerman (2002) suggested that bark should arrive at the factory and be processed on the same day as harvesting. However, this is not always possible. The simulated ageing experiment identified that light exposure had no effect, as proposed by Porter (2012). It is a common practice for bark bundles to be assembled with the cambium facing inwards (Havemann, 1992). Therefore, the effects on color and tannin content caused by the temperature and moisture interactions are mitigated by the bundle assembly, keeping the bark as dry and cool as possible. In addition, because moisture had a positive impact on the extractives and tannin content, deliberate wetting of bark during transportation or storage would increase extractives quantity but would reduce color quality. However, such batches could be used for products where color quality was not important.

Lastly, the effect of seasonal variation on factory bark samples was confounded by the differences in bark quality between the three factories over the same period (Figures 15 and 16). This is due to differences in the geographical location of factories and the site effects on the bark sources (Sherry, 1971).

4.6 Conclusion

This research confirmed the importance of several areas of research for bark quality improvement that had been previously proposed (Elmer *et al*,1964). While seasonal variation and the ageing of the bark contribute the most to color variation in the bark, their economic impact has not been quantified. Table 4.6 below presents a concise

summary of the factors influencing bark quality, their respective impacts, and potential mitigation strategies designed to optimise yield while preserving or enhancing quality

Table 4.6 A summary of the factors affecting bark quality, impacts, and mitigation options.

Factor	Impact	Mitigation Options
• Low Phosphorus (P)	• Darker Color • Impact on yield to be investigated	• Add P fertilizer to improve color quality
• Processing delays	• Darker color • Reduces Extractives	• Process bark on the same day as harvesting
• Moisture Content	• Affects extractives • Affects color inversely as bark dries out	• Ensure controlled drying to balance extractive quantity and color
• Temperature, storage, and transportation	• Darker color	• Store bark in cool, dry conditions
• Seasonal Variation	• Fluctuations in extractives and color	• Optimise harvest timing to capture peak quality
• Soil and bark nutrient availability	• Possible influence on all bark quality parameters	• Conduct multi-site studies to explore soil-bark nutrient relationships
• Climate	• Influence on all bark quality parameters	• Monitor quality shifts across multi-site studies
• Oxidation (Air) (exposure not investigated in this study)	• Effect on bark color quality	• Promote quicker processing • Investigate further

Future research can be directed at investigating the magnitude of these impacts and finding low-cost strategies to enhance the profitability of the wattle bark industry. Exploring the site effects in short and long-term studies over multiple sites where wattle is grown, along with soil, climate, and bark data could provide the industry with

tools and strategies to optimise wattle silvicultural practices for both wood and bark production to ensure higher timber volumes, as well as higher quantity and quality of the bark extract products. The effect on bark quality due to exposure to oxidation (air) was not investigated in this study but the impact is profound. Oxidation alters the phenolic chemistry of the bark and converts tannins to reactive quinones, reducing the quality (Beard, 1957). Mitigation strategies should be investigated for a more holistic approach into factors that affect wattle bark quality.

4.7 References

Anderson, M.J. 2014. Permutational multivariate analysis of variance (PERMANOVA). *Wiley Stats Ref: Statistics Reference Online*,1-15. (Accessed January 2023).

Avadianund Bridglall, P., Laing, M., Naidoo, N., and Burgdorf, R. 2025. A novel laboratory method for the extraction of black wattle (*Acacia mearnsii* de Wild.) bark constituents. *Journal of American Leather Chemists.* 120(10), 26-41.

Barnet, Y.M., Catt, P.C., and Hearne, D.H. 1985. Biological nitrogen fixation and root-nodule bacteria (*Rhizobium* sp. and *Bradyrhizobium* sp.) in two rehabilitating sand dune areas planted with *Acacia* spp. *Australian Journal of Botany*, *33*(5), 595 - 610.

Beard, J.S. 1957. *A Manual for Growing Wattle.* Natal Tanning Extract Company Limited, Pietermaritzburg. Pp 1-11.

Bray, R.H. and Kurtz, L.T. 1945. Determination of total, organic, and available forms of phosphorus in soils. *Soil Science*, *59*(1), 39-46.

Chan, J.M., Day, P., Feely, J., Thompson, R., Little, K.M., and Norris, C.H. 2015. *Acacia mearnsii* industry overview: status, key research, and development issues. *Southern Forests: A Journal of Forest Science*, *77*(1), 19-30.

Chaunbi, G. 1997. Black wattle plantations in South Africa: Introduction, silviculture, and management. In: *Black Wattle and its Utilisation*. Research Institute of Subtropical Forestry, Chinese Academy of Forestry Fuyang, China. Pp 28 - 39.

Donkin, M.J., Pearce, J., and Chetty, P.M. 1993. *Methods for Routine Plant Analysis in the ICFR Laboratories.* Bulletin Series 6/93, ICFR, Pietermaritzburg.

Dunlop, R.W. and MacLennan, L.A. 2002. Chapter 1: Introduction, Black *Wattle: The South African Research Experience*. Institute for Commercial Forestry Research. Pp 1-5.

Dunlop, R.W., Barnes, R.D., Nixon, K.M., and Hagedorn, S.F. 2003. A breeding strategy for *Acacia mearnsii* (black wattle) in South Africa: management paper. *Southern African Forestry Journal*, 199, 55-63.

Elmer, J.L., Gosnell, J.M. and Smith, R.G. 1964. A consideration of factors affecting quality in wattle bark. *East African Agricultural and Forestry Journal*, *29*(4), 350-361.

Engelmann, M.D., Bobier, R.T., Hiatt, T., and Cheng, I.F. 2003. Variability of the Fenton reaction characteristics of the EDTA, DTPA, and citrate complexes of iron. *Biometals*, *16*(4), 519-527.

Germishuizen, I. 2022. Personal communication with Dr I. Germishuizen. Research Director, ICFR, Pietermaritzburg, May 2022.

Giannotas, G., Kamperidou, V. and Barboutis, I., 2021. Tree bark utilization in insulating bio-aggregates: a review. *Biofuels, Bioproducts and Biorefining*, *15*(6), pp.1989-1999.

Gordon-Gray, C.G. 1965. The development of bark sampling from trees and plots in wattle plantations. ICFR annual research report, Pietermaritzburg. Pp 36-39.

Haase, R.F. and Ellis, M.V. 1987. Multivariate analysis of variance. *Journal of Counselling Psychology*, *34*(4), 404.

Hagerman, A.E., 2002. The Tannin Handbook. Department of Chemistry and Biochemistry. Miami University, USA. Pp 1-6.

Havemann, D.A.1992. *The Manufacture and Uses of Wattle Bark Extract.* PhD thesis, University of Stellenbosch. Pp 17-48.

Hemwall, J.B. 1957. The fixation of phosphorus by soils. *Advances in Agronomy*, *9*, 95-112.

Herselman, J.E. 2007. *The Concentration of Selected Trace Metals in South African Soils.* PhD thesis, University of Stellenbosch. Pp 4-13.

Hillis, W.E. 1997. Tannin chemistry. In: *Black Wattle and its Utilisation*. CSIRO Forestry and Forest Products, Australia. pp*106* - 111.

Hu, H., Westover, T.L., Cherry, R., Aston, J.E., Lacey, J.A., and Thompson, D.N. 2017. Process simulation and cost analysis for removing inorganics from wood chips using combined mechanical and chemical pre-processing. *Bioenergy Research*, *10*(1), 237-247.

Kassier, W.E.1959. *An Economic Analysis of Wattle Farming in the Union of South Africa*. PhD thesis, University of Natal. Pp 27-41.

Mackenzie, T.F. 1962. The wattle bark industry. *South African Forestry Journal*, *43*(1), 7-16.

Milton, S.J. 1987. Phenology of seven *Acacia* species in South Africa. *South African Journal of Wildlife Research*, *17*(1), 1-6.

Moore, P.H. and Berding, N. 2013. Flowering. *Sugarcane: Physiology, biochemistry, and functional biology*. Pp 379-410.

Nicholson, C.R.L. 1991. *Re-appraisal of the Variation of Bark Components and Quality with Age in Black Wattle Trees*. ICFR annual research report, Pietermaritzburg. Pp 221 -225.

Phillips, L. 2020. Growing success for a small-scale wattle farmer. *Farmer's Weekly*, *2020*(20037), 42-45.

Porter, L.J. 2012. Tannins. *Methods in Plant Biochemistry, 1*, 389-419.

Prentice, I.C., Cramer, W., Harrison, S.P., Leemans, R., Monserud, R.A. and Solomon, A.M. 1992. Special paper: a global biome model based on plant physiology and dominance, soil properties, and climate. *Journal of Biogeography*, 19,117-134.

Schönau, A.P.G., and Aldworth, W.J.K. 1991. Site evaluation in black wattle with special reference to soil factors. *South African Forestry Journal*, *156*(1), 35-43.

Sherry, S.P. 1971. *The Black Wattle* (*Acacia mearnsii de Wild*). University of Natal Press, Pietermaritzburg. Pp 7-30.

Sieniawska, E. and Baj, T. 2017. Tannins. *Pharmacognosy*, (10), 199-232.

Titshall, L. 2021. Residual fertiliser and liming impacts in a long-term *Acacia mearnsii* trial. *Southern Forests: A Journal of Forest Science*, *83*(1), 88-96.

Workman Jr, J.J. 2007. NIR spectroscopy calibration basics. In *Handbook of Near-Infrared Analysis*. 3rd Edition. Taylor and Francis Group CRC Press. Pp 141 – 168.

Zuur, A.F., Ieno, E.N. and Smith, G.M., 2007. Principal component analysis and redundancy analysis. In: *Analysing Ecological Data.* 1st Edition. Springer, New York. Pp 193-224.

Chapter 5: Investigations into the effects of green wattle (*Acacia decurrens* var. *dealbata* (Link) F. Muell.) bark on predominantly black wattle (*Acacia mearnsii* De Wild) bark extract quality, and its detection in mixed bark consignments

5.1 Abstract

In South Africa black wattle (*Acacia mearnsii* De Wild) and green wattle (*Acacia decurrens* var. *dealbata* (Link) F. Muell) are the only two species of wattle that are cultivated commercially. Green wattle has superior quality as a timber crop whereas black wattle produces superior tannin extracts for leather tanning applications. The cultivation of green wattle was actively discouraged by the wattle industry, until 2013 the widespread death of black wattle trees as a result of infection by wattle rust (*Uromycladium acaciae* (Cooke) P. Syd. & Syd.) inadvertently stimulated the increased production of green wattle in mixed plantations. This study aimed to determine the maximum quantity of green wattle bark that can be mixed with black wattle bark without compromising the quality of the wattle extract products. Using mixtures of green and black wattle bark ranging from 0 % to 100% of each, the quality of extracts from bark mixtures were tested for the standard SLTC quality parameters. The addition of up to 40 % green wattle bark to black wattle bark had negligible effects on the quality of bark extraction liquor. Two Near Infrared Reflectance Spectroscopy (NIRS) models were developed for wattle bark mixtures. A Qualitative (IDENT) model was developed to detect the presence or absence of green wattle bark in consignments of largely black wattle bark. A Quantitative model was developed to quantify the levels of green wattle in mixtures of green and black wattle bark. Both models were successful, allowing for the detection of green wattle bark, and the accurate prediction of the level of green wattle bark in green and black wattle bark mixtures. Using NIRS to monitor green wattle bark levels, wattle extract factories will be able to use up to 40 % green wattle bark in mixed bark consignments without compromising the quality of the tannin extracts. This will benefit wattle farmers, who will be able to increase their timber revenue without

reducing their bark revenue by increasing the percentage of green wattle to black wattle up to 40 % of each plantation.

5.2 Introduction

Several species of wattle, native to Australia, were introduced into South Africa in the 18th century. Some of the species introduced were coastal wattle (*Acacia cyclops* A. Cunn. ex G. Don), Sydney golden wattle (*Acacia longifolia* (Andrews) Wild), and golden wreath wattle (*Acacia saligna* (Labill.) H.L. Wendl.) (Stephens, 1940). In the mid–1900's, black wattle (*Acacia mearnsii* De Wild) (BW) and green wattle (*Acacia decurrens* (Link) F. Muell.) (GW) was both introduced, for both commercial and environmental purposes, such as tannin production, firewood, and to control erosion (Richardson *et al*, 2023).

The extracts of wattle bark have been utilised in the leather tanning industry for over two centuries, with the earliest documented reference to this appearing in Australian literature around 1824 (Beard, 1957). BW and GW trees can appear to be similar in the field, but they also exhibit distinctive differences. Table 5.1 below shows the visual differences between BW and GW trees (Coetzee, 1986).

Table 5.1 Visual attributes of the bark, leaves, flowers, and seeds of BW and GW Trees (Coetzee, 1986) and (https://invasives.org.za/fact-sheet/, and Moffett and Nixon, 1974).

	Black Wattle (BW)		Green Wattle (GW)	
Appearance of the bark	Light grey to light brown. Smoother outer appearance.	**Outer appearance** 	Dark grey with visible fissures.	**Outer appearance** 
	Pale cambium may have a yellow/ reddish colouration.	**Inner cambium** 	Brown to reddish coloured cambium	**Inner cambium** 
		Bark cross section 		**Bark cross section** 
Leaves	Dark green, bipinnate, finely textured leaves		Light green, feathery or fern-like textured leaves	
Flowers	Cream or white spherical flowers. Flowers between August to September		Bright yellow flowers, in round flower heads. Flowers between July to August.	
Seeds	Small, oblong shiny black or brown seeds. Seed ripening in 12 to 14 months		Slightly larger than BW. Dark brown pods. Seed ripening in 4 to 6 months	

Visual attributes have been used in the identification of BW and GW bark. The cambium colour of freshly harvested bark is a good predictor of the species of wattle

(Dunlop and MacLennan, 2002). The cambium of GW has a pink tinge, compared with the pale yellow of BW cambium.

Apart from the visual differences, BW and GW also exhibit many chemical differences. In the timber industry, GW is preferred to BW because it has many favorable qualities such as straight stem form and good wood density (Coetzee, 1986). However, in the leather tanning industry, GW is less desirable because it produces less tannins than BW, and these extracts tan leather a darker colour than BW bark extracts (Glueck,1952).

To understand these tannin quantity and colour issues, the composition of BW and GW extracts have been investigated. Reid *et al*. (2013) used electrospray ionization mass spectroscopy (ESI-MS), which showed that there are subtle differences between BW and GW bark extracts in the quantities of various dimers, trimers, and tetramers in tannin oligomers, as shown in Figure 5.1.

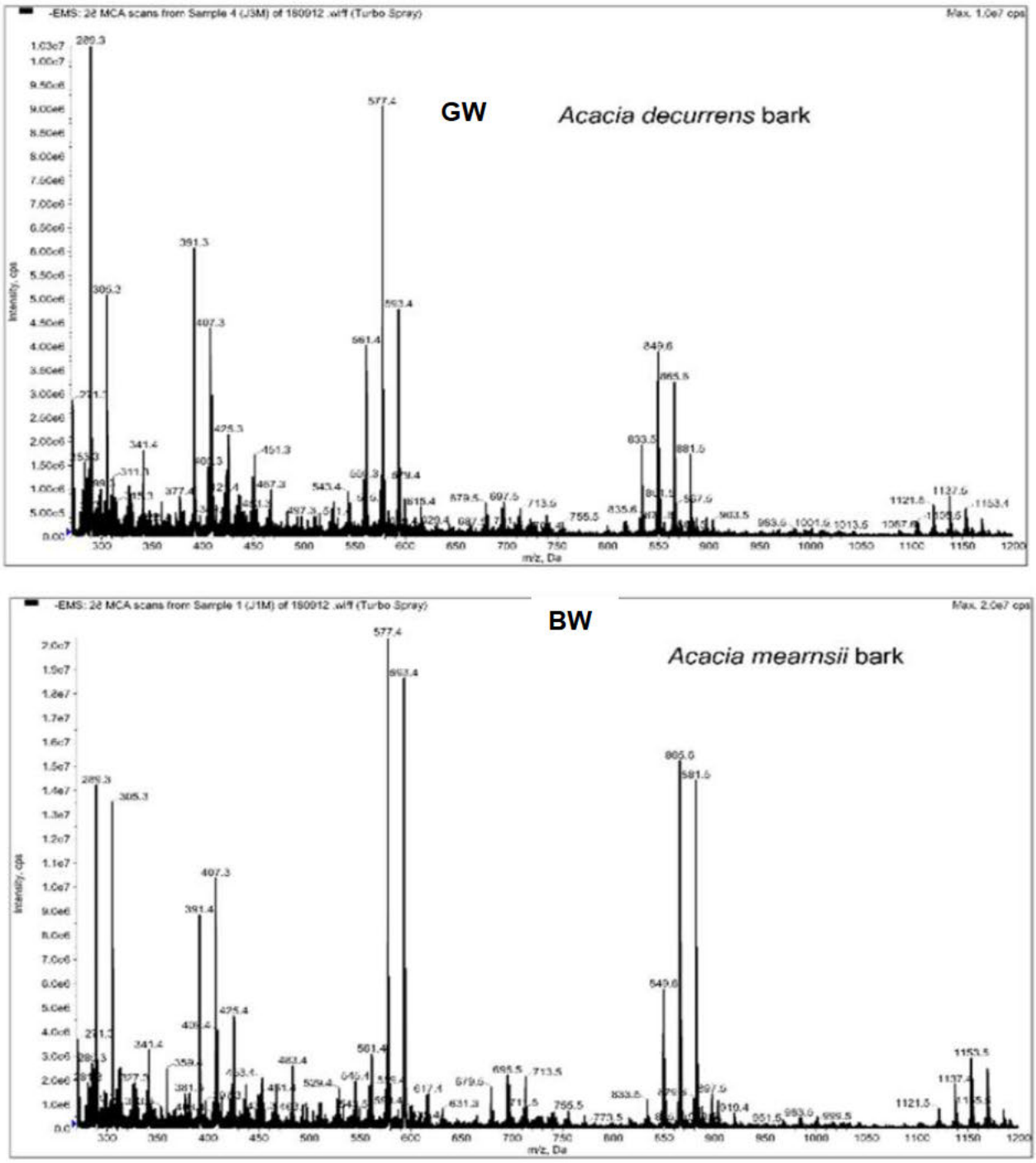


Figure 5.1 Negative ion ESI-MS chromatograms of combined methanol and acetone-water extracts of BW and GW bark (Source: Reid *et al.,* 2013, Supplementary data file).

The ESI-MS data from Reid *et al.* (2013) shows that while BW and GW produce molecules of similar size, they occur in different ratios (Sakai, 2000).

Tindale and Roux (1969) found that GW tannins gave a higher “gelatin number” than BW tannins, indicating a greater astringency. This suggests that, instead of binding to the proteins, GW tannins precipitate the leather proteins and form weaker bonds, which result in sub-standard leather, which may appear blotchy or with undesirable specks on the surface.

Although GW possessed favourable timber qualities, its cultivation was prohibited by the South African government in 1940 due to the inferior quality of its bark extracts, specifically their lower tannin content and darker colour (Craib, 1941). As a result, BW became the sole wattle species cultivated commercially, for both its timber and bark.

However, in 2013, the wattle rust (*Uromycladium acaciae*) was first observed in BW plantations in KwaZulu-Natal. The disease causes leaf and petiole lesions, defoliation, gummosis, stunting of trees and death of seedlings, raising concerns about the health and productivity of black wattle orchards. In severe cases, wattle rust has been reported to reduce the growth of young trees by 20 % to 40 % (McTaggart *et al.,* 2015). Due to the greater resistance of GW to wattle rust, there has been renewed interest in establishing GW plantations to mitigate timber losses. As timber contributes approximately 85 % of total revenue from wattle tree production, with bark accounting for only 15 % (Chan *et al.,* 2015), the shift toward GW is economically justified. However, the bark of GW remains undesirable as a primary source of quality wattle extract products due to its inferior tannin properties.

The amount of GW being sent to bark factories has not been quantified, and the future contribution of GW to the wattle bark extract industry is unclear. An improved understanding of the tannin properties of BW and GW extracts is needed to improve the management of this variable by bark extract factories.

The use of Near Infrared Reflectance Spectroscopy (NIRS) could provide a tool for the rapid and low-cost screening of wattle bark consignments sent to wattle bark factories for the presence of GW bark, and to monitor the relative levels of BW and GW being delivered to the factories.

Therefore, this research was initiated with the following objectives:

1. To identify the percentage of GW in a GW and BW bark mixture that generates a bark extract that does not compromise the quality parameters (extractives, tannin content, and Lovibond colours) of wattle bark extract products.
2. To develop NIRS models to detect the presence or absence of GW bark in consignments of wattle bark; and to quantify the relative levels of each species in bark mixtures.

5.3 Materials and methods

This work was carried out at the Institute for Commercial Forestry Research (ICFR) between 2020 and 2023.

5.3.1 Site characterisation

Three plots of ten-year-old BW and GW trees were classified at the Harden Heights plantation (Figure 5.2 below).

Figure 5.2 The aerial view of the Harden Heights Plantation (GPS coordinates - 29.2667° S, 30.6167° E). (Google Maps).

A total of 200 bark samples were taken. The diameter-at-breast height (DBH) of each tree was measured at a height of 1.3 m using a measuring tape. The percentages of BW, GW and dead trees were recorded per plot. Twenty trees from

Plot One and from Plot Two were randomly measured to determine the tree height using a Vertex Hypsometer (Haglöf, Sweden).

5.3.2 Bark Sample collection

Twenty individual fresh bark samples of BW and GW were collected from randomly selected mature trees, aged 9–10 years, at the Harden Heights plantation in KwaZulu-Natal (GPS: 29.2667° S, 30.6167° E). Bark was removed by ringbarking at a DBH of 1.3 m using a stainless-steel cleaver to minimise iron contamination, which is associated with changes in tannin colour (Slabbert, 1992). Each stripped bark sample weighed between 200 and 300 g. The samples were placed into plastic bags, vacuum-sealed, and stored in a cooler box with ice blocks to maintain freshness. They were transported to the laboratory within two hours, for further processing.

5.3.3 BW and GW sample preparation

A Vernier calliper was used to determine the thickness of each piece of bark taken, using the mean of the thickness of the piece of bark, taken on the four sides. The bark samples were weighed to determine their wet mass and freeze-dried using a Virtis BT Pro Series freeze dryer (Lab 1st, USA), set at –70 °C and 175 millitorr (mTorr) for 48 hours until a constant mass was achieved. The final dry mass was recorded to calculate the moisture content.

Once dried, all bark samples were milled to a uniform particle size of 0.5 mm using a ZM200 Retsch mill (Retsch, USA). The freeze-dried, milled bark samples had a moisture content of less than 1%. To maintain sample integrity and prevent degradation, the milled material was stored at –20 °C until further analysis.

The thickness of the bark and the recorded moisture for the BW and GW was compared using a single factor analysis of variance (ANOVA) in Excel (2025, version 2505 (Build 18827.20150)).

5.3.4 Wattle bark analysis

BW and GW bark samples were analysed for tannin content and Lovibond colour and for their mineral contents.

5.3.4.1 SLTC analysis of the bark samples

The freeze-dried, milled BW (n = 25) and GW (n = 15) bark samples were extracted using the double autoclave water (DAW) method for bark extraction (Avadianund Bridglall *et al.,* 2024). The resulting extract solution was analysed using standard methods from the Society of Leather Technologists and Chemists (SLTC) to determine total extractives, tannin content, non-tannin content, insoluble components, and Lovibond Red and Yellow colour values.

5.3.4.2 Analysis of Micronutrients and Macronutrients using MP-AES

Quantitative analysis of macronutrients and micronutrients was done on the freeze-dried, milled BW (n = 25) and GW (n =15) bark samples. The samples were first ashed in a muffle furnace (Nabertherm, China) and then digested using 16% hydrochloric acid. Calibration standards were prepared from certified reference materials obtained from De Bruyn Spectroscopic Solutions (South Africa) for the following elements:

- Macronutrients: Aluminium (Al), Boron (B), Copper (Cu), Iron (Fe), Manganese (Mn), Phosphorus (P), and Zinc (Zn).
- Micronutrients: Calcium (Ca), Potassium (K), Magnesium (Mg), and Sodium (Na).

Elemental concentrations were determined using an Agilent 4100u Microwave Plasma–Atomic Emission Spectrometer (MP-AES; Agilent, USA), with results reported in mg kg^{-1} based on standard calibration curves.

Additionally, total carbon (C), nitrogen (N), and sulphur (S) contents were measured using a Leco Trumac CNS Analyzer (Leco, USA).

5.3.4.3 Determination of the effects of various levels of GW contamination in BW bark on the quality characteristics of wattle bark extract liquor.

Samples of freeze-dried, milled BW and GW bark, (Section 5.3.2) were mixed (on a mass basis) into a graded series of mixtures, as described in Table 5.2 below. For each mixture, five different samples were made.

Table 5.2 The mixtures of BW and GW bark that were used to test bark extract quality.

Mixture ID	GW Content of the Bark Mixtures	% BW	% GW
1	0% (Pure BW)	100	0
2	10%	90	10
3	20%	80	20
4	30%	70	30
5	40%	60	40
6	50%	50	50
7	60%	40	60
8	70%	30	70
9	80%	20	80
10	90%	10	90
11	Pure GW	0	100

Bark extraction was carried out using the DAW method (Avadianund Bridglall *et al.,* 2024) and the turbidity of the extracts was determined using an A-201 Infrared Photometer Lovibond turbidity meter (HACH, South Africa) calibrated within the range of 20 to 1000 Nephelometric Turbidity Units (NTUs). This was done to determine the homogeneity of the samples. The mean, standard deviation, and coefficient of variance (CV %) were calculated to determine sample homogeneity. In laboratory assays, a sample with a CV % of < 5 % and a standard deviation of < 10 % is considered to have good homogeneity (Thompson *et al.,* 2006).

Once the homogeneity of the samples was determined, the SLTC assays were carried out on all of the samples above, with a total of 55 tests. Means of data were evaluated using the last significant difference (LSD) test to determine which bark mixtures created measurably different bark extract products. The LSD plots were created in R Studio (Cran, version 2025.05.1+513), using agricolae and ggplot2.

5.3.4.4 NIRS Quantitative Model

A NIRS Quantitative model was developed. The mixtures of BW and GW bark were scanned in triplicate on a Bruker MPA FT-NIR spectrometer (Bruker, USA) at a

controlled temperature of 20˚C, generating 55 scans in triplicate (5 scans x 11 mixtures). The triplicate scans of 49 samples (90 %) of the 11 mixtures of BW and GW bark were processed in OPUS QUANT (v8.5, Bruker, Germany). The model used the percentage of GW in each mixture as the reference data. Data processing was conducted within in the OPUS QUANT environment using Partial Least Squares Regression (PLSR). Spectral pretreatment included vector normalisation and appropriate NIR preprocessing procedures to enhance signal quality and improve model robustness.

Model performance was evaluated using R^2, RMSEP, and RPD, which assess how well the NIRS model aligns with reference data. Additionally, RPIQ was calculated as the interquartile range (IQR) divided by RMSEP to test model robustness.

After developing the NIRS Quantitative model, an independent test set comprising of the remaining six samples (10 %) of the known bark mixtures were used to evaluate the model's predictive performance. Analysis was done using the Quant Analysis module in OPUS (version 8.5, Bruker, Germany). The model accuracy was assessed by comparing predicted values with the reference values.

5.3.5 Factory bark sample collection

A total of 56 chopped and chipped bark samples were collected separately from the three existing wattle bark extract factories in South Africa. The samples were taken directly from bulk loads on the conveyor belts, where wattle bark is mixed during the chopping and chipping processes. Upon arrival at the ICFR, the samples were freeze-dried and milled. The freeze-dried, milled bark samples were then stored at -20°C to maintain their chemical integrity.

These samples were analysed using the NIRS Quantitative model developed to evaluate the model's ability to detect whether any GW has been mixed with the BW. The samples were also analysed for their SLTC bark quality parameters.

5.3.6 NIRS Qualitative (IDENT) model

An IDENT model was created to evaluate whether NIRS could differentiate between pure BW and GW bark. The pure BW and GW bark were scanned in triplicate on a Bruker MPA FT-NIR spectrometer (Bruker, USA) at a controlled temperature of 20˚C. The spectra were analysed using the OPUS IDENT software (version 8.5,

Bruker, Germany). All the scanned samples were uploaded and named after their specific percentage of GW The spectral region was determined between 3594 – 14981, and data preprocessing was applied using 1st derivative and Vector Normalization. A 2D and 3D score plot was created.

5.4 Results

5.4.1 Site data

Table 5.3 shows the data of the three plots that were characterized for the sampling during the 2020/2021 period. There was a mean mortality of 45 %, indicating poor site quality, and 14 % of trees within the stand were identified as GW. The GW trees tended to be larger than the BW trees. In the stands that were sampled, GW trees had a mean height of 17.8 m, which was 7 % taller than the mean height of the BW trees, and had a mean DBH of 14.0 cm, which was 6 % wider than the mean of the BW trees.

Table 5.3 The volume of BW, GW, and dead wattle trees at Harden Heights, average tree DBH, and mean tree height of three plots.

Measurements	Plot 1	Plot 2	Plot 3
% BW trees	50.0	29.0	41.5
% GW trees	6.0	16.0	20.5
% Dead trees	44.0	55.0	38.0
Mean tree height BW (m)	16.4	17.3	16.1
Mean tree height GW (m)	18.2	17.3	17.9
Mean DBH BW (cm)	12.1	15.6	11.8
Mean DBH GW (cm)	13.3	13.9	14.7

5.4.2 Comparison of Bark Thickness and Bark Moisture Percentage

The bark thickness was compared between BW and GW trees; Table 5.4 shows the results from the Single Factor ANOVA used for statistical comparison. The BW bark was 5 % thicker than the GW bark, but the p-value was greater than 0.05, indicating that the difference was not significant statistically.

Table 5.4 Single Factor ANOVA of bark thickness for BW and GW bark samples.

Groups	Count	Sum	Average	Variance		
BW	20	8.38	0.42	0.03		
GW	20	8.00	0.40	0.02		
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	0.01	1.00	0.00	0.14	0.71	4.10

Table 5.5 shows the single-factor ANOVA for moisture content of BW and BW samples taken. BW had a higher moisture % than GW, but the variation between the samples for BW was double that of GW. The p-value is less than 0.05, indicating a significant difference in moisture between BW and GW.

Table 5.5 Single Factor ANOVA of moisture % for BW and GW bark samples.

Groups	Count	Sum	Average	Variance		
BW	20	859.11	42.96	14.69		
GW	20	804,59	40,23	7,37		
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	74.31	1.00	74.31	6.74	0.01	4.10

5.4.3 Comparison of Bark Quality Parameters

The bar plot in Figure 5.3 shows a comparison of bark quality parameters (SLTC) of BW and GW. The mean values of extractives %, tannin % and non-tannin % of the BW bark were 30.7 %, 49.9 % and 23.9 % greater than those of the GW bark, respectively, with the standard deviation shown with error bars, BW has overall lower standard deviations than GW for all bark quality parameters. The GW bark had 107% more insoluble products than the BW bark. The Lovibond colour was almost two times darker for GW bark extracts than BW bark extracts.

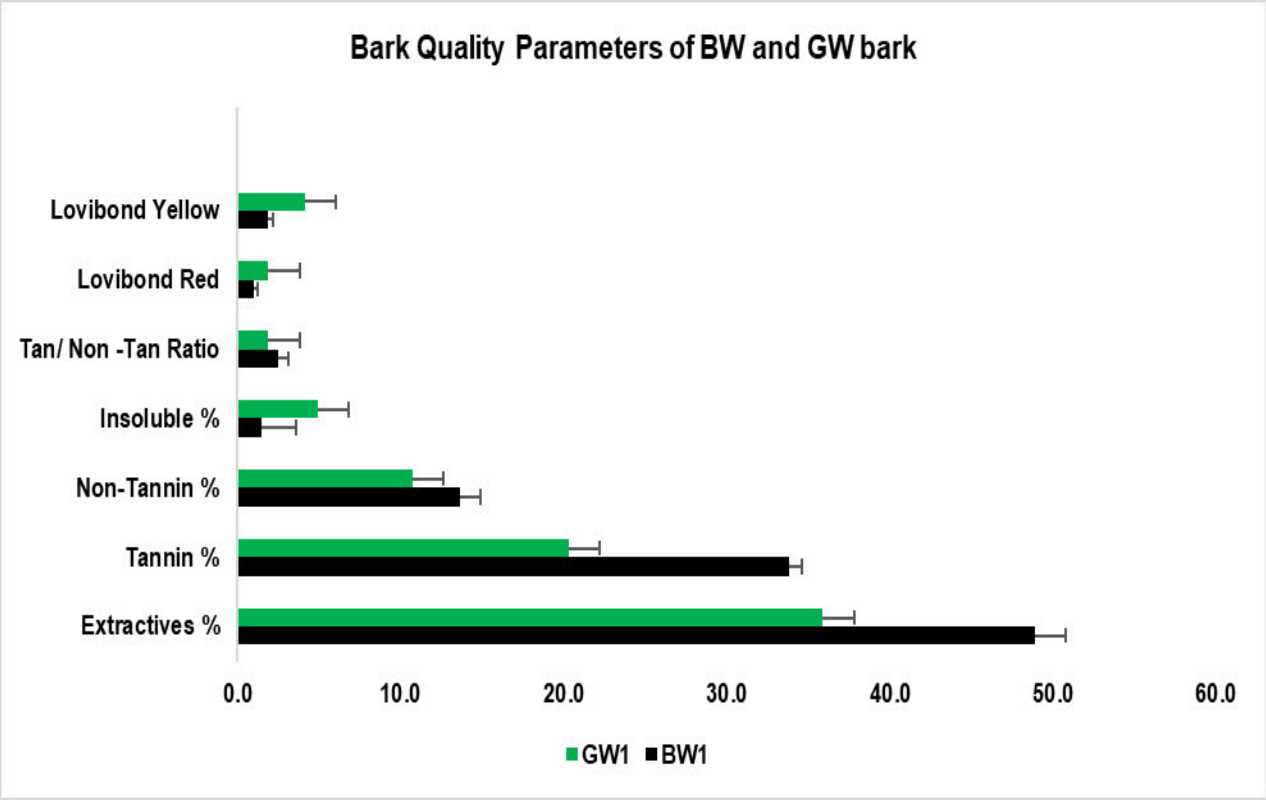


Figure 5.3 Plot of bark quality parameters (Extractives %, Tannin %, Non-tannin %, Insoluble %, Tan/ Non-Tannin Ratio, Lovibond Red, and Lovibond Yellow), comparing BW and GW bark extracts. The error bars show the standard deviation.

5.4.4 Comparison of Bark Minerals

The comparison of minerals in Figure 5.4 showed that BW bark had more Ca, Al, Fe, and P, whereas GW bark had more K and Mn. The rest of the minerals were relatively similar in BW and GW bark. The standard deviation shown in error barks did not show significant differences.

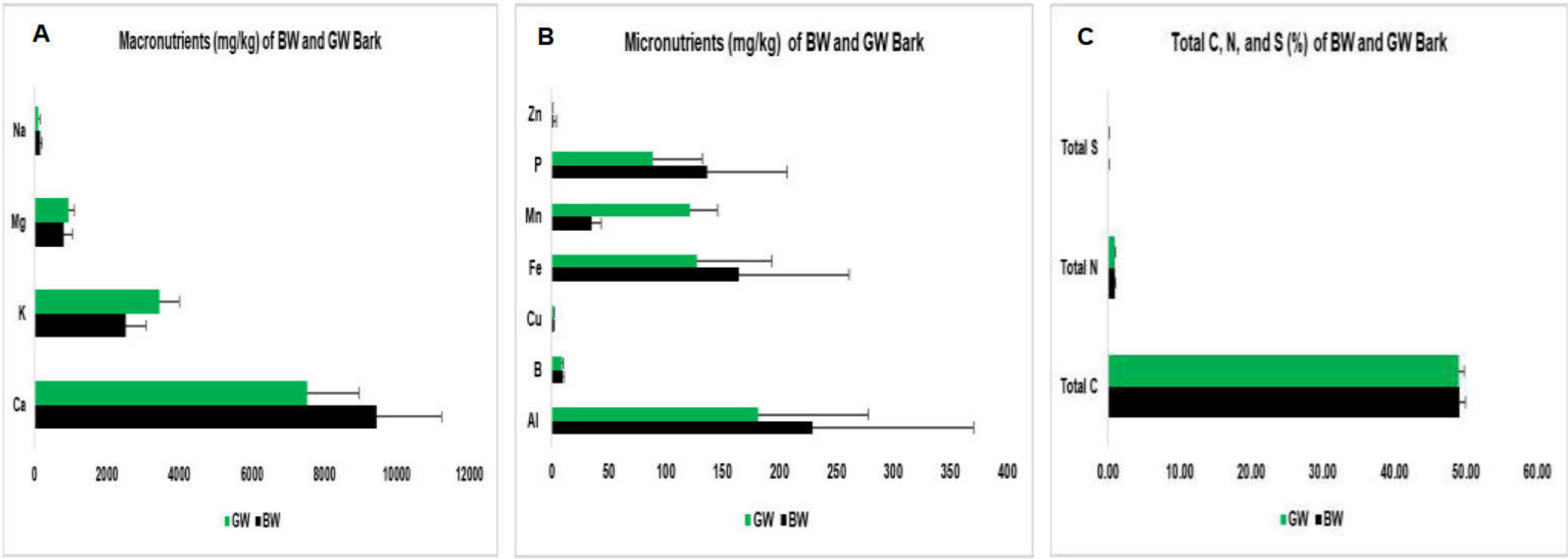


Figure 5.4 Plots of (A) Macronutrients (Ca, K, Mg, Na in mg/kg), (B) Micronutrients (Al, B, Cu, Fe, Mn, P, and Zn in mg/kg), and (C) Total C %, N %, and S % for BW and GW bark. Error barks show the standard deviation.

5.4.5 Analysis of BW and GW bark mixtures

5.4.5.1 Homogeneity testing of BW and GW mixtures

Table 5.6 presents the results of the turbidity analysis for the homogeneity assessment conducted on each sample prepared across the different bark mixtures. Consistently low standard deviation and CV % values confirmed that all the samples were homogeneous and suitable for analysis.

Table 5.6 Turbidity testing of the BW and GW bark mixtures prepared to determine the Mean, Standard deviation, and CV % for homogeneity.

Mixture ID	GW% in Mixtures	Mean (Ntu)	Standard Deviation	CV %
1	0%	189.1	5.4	2.8
2	10%	208.4	5.3	2.6
3	20%	220.4	3.1	1.4
4	30%	226.9	2.7	1.2
5	40%	230.3	3.9	1.7
6	50%	229.0	5.3	2.3
7	60%	248.9	5.2	2.1
8	70%	259.3	5.7	2.2
9	80%	279.2	2.7	1.0
10	90%	291.5	2.3	0.8
11	100% GW	312.7	3.1	1.0

5.4.5.2 SLTC analysis of the BW and GW mixtures

Figure 5.5 displays the visual appearance of the mixtures extracted from the eleven samples created (100 % BW bark, 100 % GW bark, and nine mixtures of BW and GW bark). There was a subtle change in the hue of the extracted mixtures. The extracts from 100 % BW, 10 % GW, and 20 % GW appeared to be much duller than the extracts from the 30 % GW, 40 % GW, 50 % GW, 60 % GW, 70 % GW, and 80 % GW mixtures. The extracts from the 90 % GW mixture and the 100 % GW sample appeared to have the darkest colour (brownish/ red).

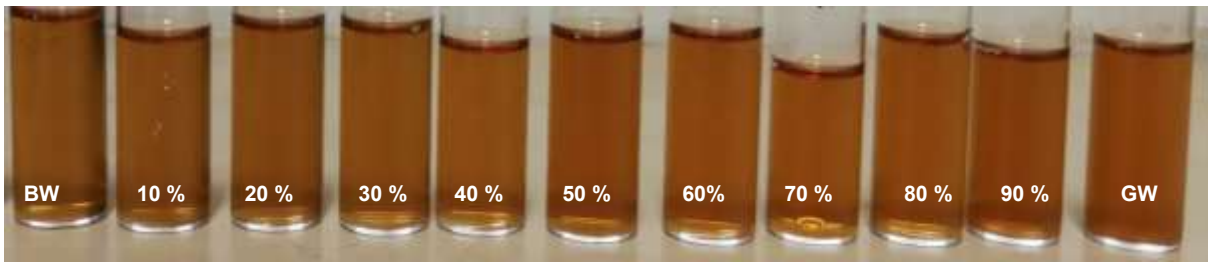


Figure 5.5 Visual appearance of the bark mixture extracts from 100% BW, 100% GW, and the different BW and GW mixtures.

The LSD plots created for the different bark quality parameters are shown, below in Figure 5.6 A – D. The LSD plot A of Extractives %, Tannin %, and Non-tannin % shows that that the 100 % BW, and the 10 % to 40 % BW and GW mixtures were similar and showed no significant differences ($p > 0.05$). However, from the mixtures with more than 50 % GW content, the LSD plots were significantly different. A similar trend was noted for the Lovibond Red colour, whereas the Lovibond Yellow colour showed significant differences between 100 % BW, 100 % GW, and all mixtures. The Insoluble % also showed significant differences between all tested samples. The Tannin/Non-Tannin ratio showed no significant difference between the extracts from 100 % BW, 10 % GW, 20 % GW, and 30 % GW (Mixtures 1-3). All other bark mixtures, and 100 % GW were significantly different.

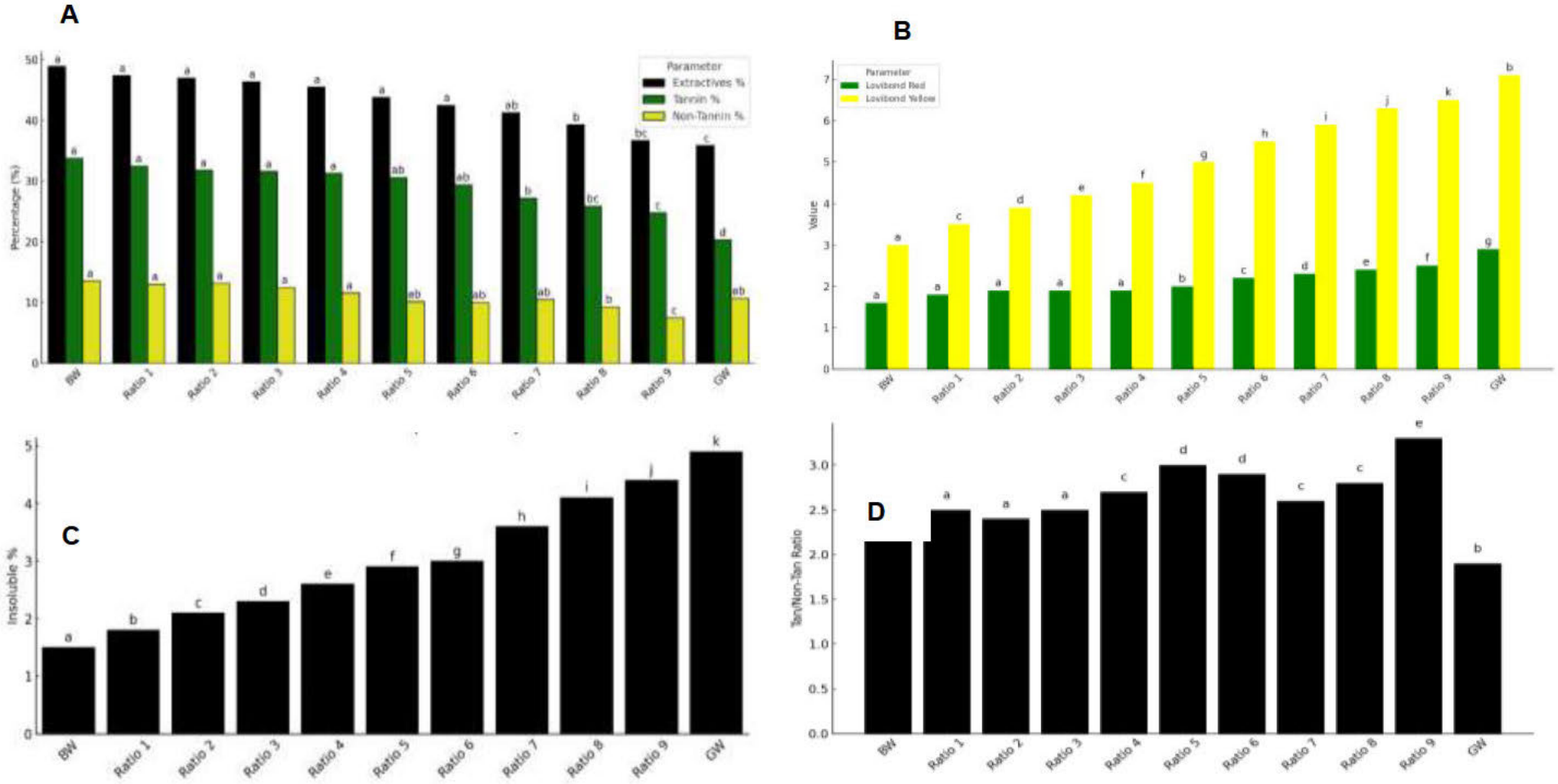


Figure 5.6 LSD plots represent the mean values for (A) Extractives %, Tannin %, and Non-Tannin %, (B) Lovibond Red and Lovibond Yellow, (C) Insoluble %, and (D) Tannin/Non-Tannin ratio for 100% BW, 100% GW and the nine BW and GW bark mixtures. Different letters mean that differences were significant (P </= 0.05).

5.4.5.3 NIRS Quantitative model

The calibration and validation results for the NIRS Quantitative model, developed using 49 of the samples comprising of BW, GW, and the BW and GW mixtures, is presented in Table 5.7. Both the calibration and validation models exhibit high R^2 values. The high RPIQ further confirms the model's strong predictive accuracy, while the high RPD and low RMSEP values indicate that the model is both reliable and robust.

Table 5.7 NIRS Quantitative model of bark mixtures ranging from pure BW to pure GW, with nine combinations of BW and GW bark in 10% increments.

Parameter	Range (Min)	Range (Max)	Spectral Range	Calibration		Validation		RMSEP	IQR	RPIQ
				R^2	RPD	R^2	RPD			
Mixture	0	10	4242.8 - 7506	99.9	29.7	99.8	22.2	0.138	4.1	30.0

5.4.5.4 Independent test set for NIRS Quantitative Model

Six samples with known BW and GW mixtures were used to evaluate the model's predictive accuracy for bark composition. The results of this independent test set are presented in Table 5.8. The NIRS Quantitative model successfully predicted the different variants with minimal standard deviation, demonstrating its effectiveness as a reliable tool for qualitative screening of bark samples.

Table 5.8 Independent test set of the NIRS known values (assigned sample ID's) versus the average NIRS predicted value.

Sample ID	Actual % of GW bark	NIRS predicted value	Standard deviation
Pure BW	0	0	0
Pure GW	100	100	0.121
Mixture 3	20	20	0.355
Mixture 5	40	40	0.098
Mixture 7	60	60	0.071
Mixture 9	80	80	0.074

5.4.5.5 NIRS Quantitative model tested on factory samples

Of the 56 factory bark samples screened using the NIRS Quantitative model, only eight samples (14 %) produced predicted values for the BW:GW mixtures of greater than 0.5. Most of the percentages predicted for the samples fell within the 0 to 0.5 ratio. Table 5.9 presents the predicted BW:GW mixtures, actual SLTC data (Tannin % and Lovibond Red), and the corresponding SLTC values based on the predicted ratios. Analysis of the percentage differences indicated that the model's predictions were primarily influenced by Tannin %, which showed the lowest variation. Lovibond Red values closely matched the actual data, with percentage differences ranging from 0 % to 11.8 %.

Table 5.9 Factory dataset scanned using the NIRS Quantitative model to predict the percentage of GW in BW and GW bark mixtures. Values included are actual SLTC data, SLTC parameters based on the predicted GW content of the mixtures, and the percentage difference between the two.

Sample	% GW Predicted	*Closest Mixture	Mixture SLTC Values		Actual SLTC Values		% Difference	
			Tannin %	Lovibond Red	Tannin %	Lovibond Red	Tannin	Lovibond Red
A	8	Mixture 1	32.5	1.8	32.1	1.6	1.2	11.8
B	10	Mixture 1	32.5	1.8	32.8	1.6	1.0	11.8
C	38	Mixture 4	31.3	1.9	30.4	2.1	2.9	10.0
D	14	Mixture 1	32.5	1.8	34.0	1.9	4.5	5.4
E	14	Mixture 1	32.5	1.8	33.1	1.9	1.8	5.4
F	9	Mixture 1	32.5	1.8	32.5	1.6	0	11.8
G	8	Mixture 1	32.5	1.8	32.9	1.8	1.2	0
H	18	Mixture 2	31.8	1.9	31.4	1.8	1.3	5.4

* The NIRS predicted values for % GW (Column 2) were rounded off to the closest % of GW used in the mixtures (from mixture 1 containing 0 % of GW to Mixture 11 containing 100 % of GW)

5.4.5.6 NIRS Qualitative (IDENT) model

Figure 5.7 shows the (A) 2D and (B) 3D score plots for the 100% BW, 100% GW and the nine mixtures. The tight clustering indicates the close similarities of the samples. The yellow sphere representing 100 % GW was not linked to the rest of the spheres.

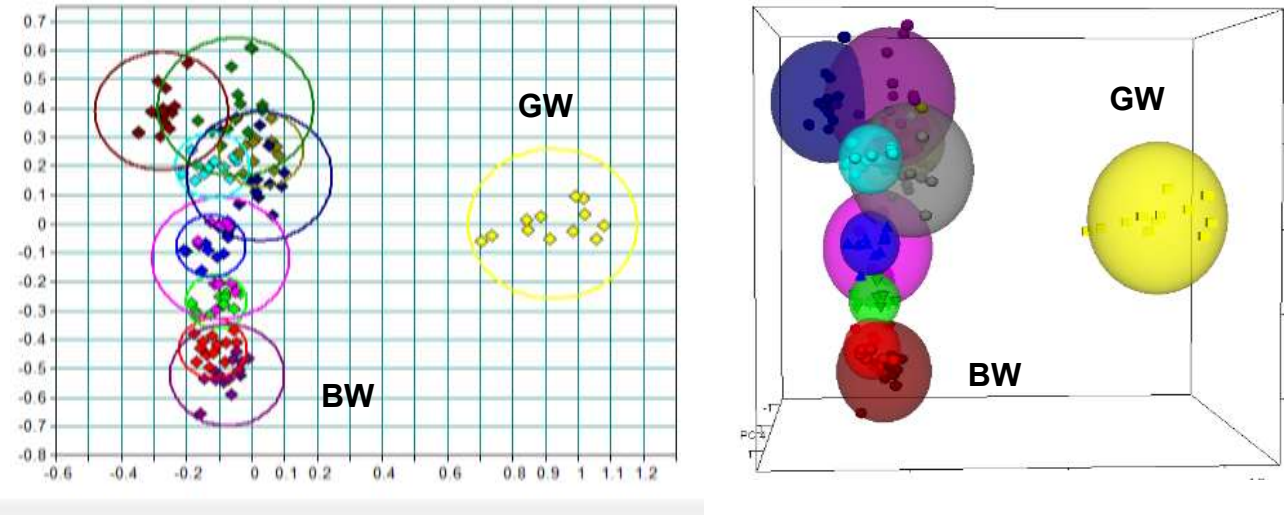


Figure 5.7: NIRS Ident (A) 2D and (B) 3D score plot used to qualitatively identify varying levels of BW, GW, and different mixtures of BW and GW.

5.5 Discussion

This study focused on evaluating BW and GW trees and their bark, starting with the site characterisation of the Harden Heights plantation. The Harden Heights plantation exhibited a tree mortality rate of 46 %, with 56 % of the trees remaining alive, of which 2 5% were identified as GW trees. Given the high mortality rate, the site is considered to be of poor quality. However, the elevated mortality and notable presence of GW may have been linked to the emergence of wattle rust in 2013 because GW has greater frost tolerance and rust resistance than BW, with frost and rust being the two primary causes of wattle tree mortality (Chan *et al.,* 2015). The GW trees were found to be taller and wider than the BW trees on the site. However, the thickness of the BW and GW bark showed no significant differences. The moisture content of BW was 7 % higher than that of GW. Bark moisture plays an important role in wattle bark extract quality because it affects extraction yield, tannin concentration, and the efficiency of the extraction process. Dry bark is brittle and has less tannin content and darker colours, which affect bark quality parameters (Hillis, 1997).

The comparison of BW and GW bark quality parameters (Figure 5.3) supports the previous findings that BW bark has better SLTC bark quality parameters than GW (Ogawa and Yazaki, 2018). BW showed higher extractives and tannin content,

combined with lower non-tannin and insoluble fractions. The Lovibond colour of 100 % BW bark extract was lower than that of the GW extract, where less colour is desirable for the tanning industry's requirements (Dunlop and MacLennan, 2002). The BW bark extract had 3 0% more extractives, 50 % higher tannin content and a 60 % better colour than GW bark extract.

BW bark contained higher levels of bark minerals such as Ca, Al, Fe, and P, whereas GW bark had higher concentrations of K and Mn. An interaction of Al, Fe, and P levels in wattle bark has been associated with the colour of wattle bark extracts, where low levels have been linked to darker colouring (Hemwall, 1957 and Avadianund Bridglall *et al.,* 2025). High levels of Mn in the bark have been associated with dark colour development, caused by the disruption of the phenolic biosynthesis pathways (Dunlop and MacLennan, 2002).

Due to the potential impact of GW bark on mixed wattle bark extract quality, the wattle industry needs to be able to quantify the GW content of bark consignments from wattle plantations. The wattle bark to timber ratio is an average of 30:70 % per hectare of trees planted. The current value of wattle timber is R1,550 per ton, whereas raw wattle bark is valued at between R1,900 to R2,600 per ton, depending on quality or grade (Per's communication, Tomlinson, 2025). If a wattle farmer were unable to sell the bark of a wattle plantation due to a high level of GW trees, this would translate into an average loss of R30,000 per hectare. The financial losses for the wattle extract factories would be substantial if high levels of GW bark were to be mixed with BW bark, resulting in poor quality bark extracts.

However, the question of whether a small proportion of GW bark could be mixed with BW bark without affecting bark extract qualities has not been systematically evaluated. To do so, various mixtures of BW and GW bark were prepared in the laboratory to evaluate the feasibility of using mixtures of BW and GW bark. Homogeneity testing confirmed that the samples were well mixed and revealed that turbidity increased with higher proportions of GW, indicating a greater presence of insoluble particles in GW bark. GW usually has thicker, more fibrous periderm layers or bark tissue, containing a higher proportion of corky or suberised cells, which contribute to increased levels of insoluble extracts (Insoluble %) (Fraga Corral *et al.,* 2020). Further fibre testing may contribute to a better understanding this finding.

A visual comparison of bark extract liquors from the bark mixtures (Figure 5.5) demonstrated a spectrum of colours in the liquors between the extracted from the 11 samples. In the mixed bark samples, there were subtle colour changes at lower GW concentrations. The colour only became visually distinguishable in mixtures with 50 % or more of GW bark. These observations were supported by LSD plots (Figure 5.6) of bark extract quality parameters, which showed that at low GW inclusion levels, the impact on liquor quality was minimal. However, at GW concentrations of 50 % or more, the liquor quality was negatively affected, with reduced Tannin content and intensified Lovibond Red and Lovibond Yellow colours. Notably, the Lovibond Yellow values increased strongly, deviating from the typical 1:2 red-to-yellow ratio observed in BW liquors.

The Tannin/Non-tannin ratio showed no significant difference between 100 % BW and Mixtures 2 to 4 (10 %, 20 %, and 30 %of GW bark). A Tannin: Non-Tannin ratio of less than 2.4 indicates an adequate level of tannin for bark and extract powder quality (Chan *et al*.,2015). According to Sherry (1971), acceptable tannin content in mature BW bark (10-year-old tress) may range from 27.1 % to 41. 8%, with an average of 36.8 % on a moisture-free basis. Extracts from BW and GW bark mixtures with 10 % to 70 % GW bark inclusions had Tannin: Non-Tannin values of between 33.8% and 27.2%, i.e., within the acceptable range. However, when taking all bark quality parameters into consideration, a maximum of 60 % BW to 40% GW bark could be allowed at extract factories without compromising the quality of the bark extracts.

Given that the current methods of bark analysis is slow and inefficient (Gordon-Gray, 1953), NIRS evaluated in a search for a fast, scalable and reliable method to screen wattle bark consignments for the presence or absence of GW bark (Qualitative Model), and the quantity of GW present in mixed consignments of BW and GW bark (Quantitative Model). The NIRS models was created as a rapid tool to qualitatively and quantitatively predict the presence or absence of GW bark, and the level of GW bark in mixtures of BW and GW bark.

Many comparison models have been created for applications in agriculture, such as the verification of raw materials in the feed industry, for genetics and plant breeding, and for quality control and traceability (Vincent and Dardenne, 2020). The qualitative

model performed as expected, with a distinct difference between the spectra of pure BW and GW bark (Figure 5.7).

The NIRS Quantitative model performed exceptionally well, yielding nearly perfect results, as seen in Table 5.7. The model was validated using an independent test set composed of bark mixtures with known GW contamination levels. Using this model, previously analysed samples from the three wattle extract factories were evaluated, and the outcomes were highly encouraging. The predicted BW and GW percentages in the bark mixtures were mapped onto an assessment of the quality parameters of the bark extracts using conventional SLTC methods for known BW and GW mixtures. The predicted versus actual values were accurate to within 4.5 % for Tannin % and 11.5 % for Lovibond Red colour.

This method was developed under controlled laboratory conditions. The next stage in the process would be to run a pilot trial at a wattle bark processing facility where known BW and GW mixtures could be mixed, chipped, and analysed. This would confirm the reliability of the NIRS Quantitative model operating under real world conditions, at a near-industrial scale. With further refinement, this NIRS model could be used routinely at a factory level as a rapid screening tool to quantify the level of GW in each bark consignment arriving at the factory. These predictions could them be used as the basis for a blending process whereby consignments with a low GW bark content could be mixed with consignments with a higher GW content, to ensure that the 40% threshold of GW in the final blend is not exceeded, and that the quality of the bark extracts can be maintained. Further NIRS models could be developed for the prediction of quality parameters in bark extract thin liquors, thick liquors and the final extract products that are manufactured, allowing for NIRS-based quality control steps to be included at all key stages in the manufacturing of wattle bark extracts.

Further research on the bark of GW is necessary, to determine its potential use in various wattle-based products such as animal feed, water treatment chemicals, and adhesives, which remain largely unexplored. This study has demonstrated that GW can be blended with BW at appropriate levels without significantly compromising liquor quality. These findings could assist farmers who need to plant GW for the management of challenges such as wattle rust, frost, and other diseases to which BW is more susceptible. Additionally, given the decline in wattle plantation area in

South Africa (Morris, 2022), the strategic use and planting of GW could support the long-term sustainability of the wattle extract industry.

5.6 References

Avadianund Bridglall, P., Laing, M., Naidoo, N., and Burgdorf, R. 2024. A novel laboratory method for the extraction of black wattle (*Acacia mearnsii* de Wild.) bark constituents. *Journal of American Leather Chemists Association*,120(10), 26 – 34.

Avadianund Bridglall, P.A., Laing, M.L., Morris, C. and Burgdorf, R.J. 2025. Ageing, climatic, physical, and site effects on black wattle (*Acacia mearnsii* de Wild.) bark. View online at Wiley Online Library (wileyonlinelibrary.com); DOI:10.1002/bbb.2795; *Biofuels, Bioproducts and Biorefining.* 1- 17.

Beard, J.S. 1957. *A Manual for Growing Wattle.* Natal Tanning Extract Company Limited, Pietermaritzburg. Pp1-11.

Chan, J.M., Day, P., Feely, J., Thompson, R., Little, K.M., and Norris, C.H. 2015. *Acacia mearnsii* industry overview: current status, key research and development issues. *Southern Forests: A Journal of Forest Science*, *77*(1), 19–30.

Coetzee, J. 1986. A comparison of bark yield and pole utilisation between black wattle (*Acacia mearnsii*) and green wattle (*A. decurrens*).In: *Annual Report, Institute for Commercial Forestry Research, University of Natal*, pp.133-139.

Craib, I.J. 1941. South African wattle bark and wattle extract with special reference to the American market. *Journal of the South African Forestry Association*, *6*(1), pp.71-88.

Dunlop, R.W. and MacLennan, L.A. Chapter 1: Introduction, Black *Wattle.* In: *The South African Research Experience*. Institute for Commercial Forestry Research, Pietermaritzburg, South Africa. Pp 1-5, 2002.

Fraga-Corral, M., García-Oliveira, P., Pereira, A.G., Lourenço-Lopes, C., Jimenez-Lopez, C., Prieto, M.A. and Simal-Gandara, J., 2020. Technological application of tannin-based extracts. *Molecules*, *25*(3), p.614.

Glueck, L.D. 1952. *A Preliminary Investigation of the Structure of Green Wattle Tannin (Acacia decurrens Wild).* Ph.D. thesis, Rhodes University. Pp 1-10.

Gordon-Gray, C.G. 1953. A rapid method of determining the relative tannin content of young, living black wattle trees in genetic and breeding experiments. *Report-Wattle Research Institute*. Pp 42-48.

Hemingway, R.W. and Karchesy, J.J. 2012. *Chemistry and Significance of Condensed Tannins*. Springer Science & Business Media, London. Pp 517-527.

Hemwall, J.B. 1957. The fixation of phosphorus by soils. *Advances in Agronomy*, *9*, 95-112.

Hillis, W.E. 1997. Tannin chemistry. In: Brown, A.G., and Ko, H.C. (Eds): *Black Wattle and its Utilisation*. Rural Industries Research and Development Corporation, Canberra, Australia. Pp*106*.

McTaggart, A.R., Doungsa-ard, C., Wingfield, M.J. and Roux, J. 2015. Uromycladium acaciae, the cause of a sudden, severe disease epidemic on Acacia mearnsii in South Africa. *Australasian Plant Pathology*, *44*, pp.637-645.

Moffett, A.A. and Nixon, K.M. 1974. The effects of self-fertilization on green wattle (*Acacia decurrens* Willd.) and black wattle (*Acacia mearnsii* De Wild.). Wattle Research Institute, South Africa. Pp 66 – 84.

Morris, A.R. 2022. Changing use of species and hybrids in South African forest plantations. *Southern Forests: A Journal of Forest Science*, *84*(3), 193-205.

Ogawa, S. and Yazaki, Y. 2018. Tannins from *Acacia mearnsii De Wild* Bark: Tannin determination and biological activities. *Molecules*, *23*(4), 837.

Oumar, S. 2020. *Assessing the Utility of Landsat 8 Multispectral Sensor and the MaxEnt Species Distribution Model to Monitor Uromycladium acaciae Damage in KwaZulu-Natal, South Africa*. Ph.D. thesis, University of KwaZulu-Natal. Pp 1-3.

Reid, D.G., Bonnet, S.L., Kemp, G. and van der Westhuizen, J.H. 2013. Analysis of commercial proanthocyanidins. Part 4: Solid state 13C NMR as a tool for in situ analysis of proanthocyanidins tannins, in heartwood and bark of quebracho and acacia, and related species. *Phytochemistry*, *94*, 243-248.

Richardson, D.M., Binggeli, P. and Botella, C. 2023. Australian *Acacia* Species in Africa. *Wattles: Australian Acacia Species Around the World*, Pp.181-200.

Sakai, K. 2000. Chemistry of bark. *Wood and Cellulosic Chemistry, 2nd ed.* Chapter 7. Pp.243-273.

Sherry, S.P. 1971. *The Black Wattle* (*Acacia mearnsii de Wild*). University of Natal Press, Pietermaritzburg. Pp 7-30 and 40-44.

Slabbert, N. Complexation of condensed tannins with metal ions. In: *Plant Polyphenols*. Springer, Boston, MA, USA. Pp 421 – 436, 1992.

Stephens, R.P. 1940. Mining timber in South Africa with special reference to wattle silviculture. *Journal of the South African Forestry Association*, *4*(1), 55-74.

Thompson, M., Ellison, S.L. and Wood, R. 2006. The International Harmonized Protocol for the proficiency testing of analytical chemistry laboratories (IUPAC Technical Report). *Pure and Applied Chemistry*, *78*(1), 145-196.

Tindale, M.D., and Roux, D.G. 1969. A phytochemical survey of the Australian species of *Acacia*. *Phytochemistry*, *8*(9), 1713-1727.

Tomlinson, G. 2025. Personal communication with Mr. G. Tomlinson. Research and development manager, UCL, Dalton, June 2025.

Vincent, B. and Dardenne, P. 2020. Application of NIR in Agriculture. In *Near-infrared Spectroscopy: Theory, Spectral Analysis, Instrumentation, and Applications.* Springer Singapore, Singapore. Pp. 331-345.

Wingfield, M. J., and Knox-Davies, P. S. 1987. *Uromycladium tepperianum* on *Acacia mearnsii* in South Africa. *Phytophylactica*, 19(3), 287–289.

Chapter 6: Application of Near-Infrared Spectroscopy (NIRS) to estimate the bark quality of black wattle seed sources and clones

6.1 Abstract

Black wattle (*Acacia mearnsii* de Wild.) is an important and high-value South African forestry species for bark and timber production. One of the objectives of the wattle breeding programme at the Institute for Commercial Forestry Research (ICFR) is to improve bark quality. Evaluating bark quality in breeding trials requires sampling of large numbers of trees, without causing any damage to the valuable wattle trees. Therefore, traditional wet-chemistry lab methods are impractical because of they are slow, expensive and require destructive sampling. Thus, the ICFR breeding programme would benefit from a rapid method to assess bark quality with minimum damage to the trees or completely non-destructively. This study employed a novel Near Infrared Spectroscopy (NIRS) rapid and semi-destructive screening protocol to assess the quality of bark in field trials of black wattle. The protocol was used to compare wattle bark quality parameters of trees in field trials, established using various seed sources and clonal material. The predictive models developed showed a high degree of accuracy across the various seed sources and clonal varieties. The study showed that bark quality parameters across six different seed sources at a block trial in Luneburg showed very little variation, whereas bark from the same seed sources in another block trial in Iswepe (Creydt) showed differences to each other and were different to the same sources planted in Luneburg. In the clonal trials, Tannin % and Lovibond colour showed more variation at Schwarzwald, (CLT2) than at Harden Heights (CLT3), with SP1-35 being one of the best performing clones at both Schwarzwald and Harden Heights. Site-by-genotype interaction analysis revealed that certain clones performed better in specific soil conditions. These findings can assist in guiding seed source/clone selection and site matching strategies, ensuring that the genetic potential of black wattle germplasm is maximised under the most suitable growing conditions. Overall, the development of the NIRS model can contribute to a more targeted and efficient breeding process and to the sustainable development of black wattle plantations.

6.2 Introduction

Plant breeding is the practice of improving plant species to develop desirable traits such as improved quality parameters, higher yields, disease resistance, and adaptability to diverse environmental conditions (Acquaah, 2015). The process entails the selection of plants exhibiting desirable traits, followed by crossbreeding to develop new varieties or hybrids that possess superior characteristics (Moose and Mumm, 2008). Plant breeding can be done using conventional approaches such as controlled cross-pollination and selective breeding, or by employing recently developed methods such as genetic modification and molecular techniques (Gepts and Hancock, 2006) The objective of plant breeding is to develop crop varieties that are more desirable for human use, to enhance agricultural output, and to contribute to sustainable environmental practices (Hayward *et al.*, 2012).

The Institute for Commercial Forestry Research (ICFR), initially established as the Wattle Research Institute (WRI), has been involved in wattle breeding since 1947 (Dunlop, 2002). *Acacia mearnsii* (de Wild.), commonly known as black wattle, is a fast-growing tree species, native to south-eastern Australia. It is widely cultivated in regions such as South Africa and Brazil for its bark, which is rich in tannins, which are used in the leather industry, and for its wood, which is used in paper and pulp production (Griffin *et al.,* 2023). Black wattle is also known for its ecological adaptability and high wood density, making it a valuable resource for both tannin production and woodchip exports (Poynton, 2003).

Breeding programmes aim to develop new wattle genotypes with diverse favourable traits. This can strengthen genetic diversity, enabling the development of improved cultivars with enhanced tannin yields, superior wood quality, and better resilience to environmental stresses and pests (Bairu *et al*, 2021). These objectives are achieved through a combination of selective breeding methodologies, including traditional techniques such as provenance testing, vegetative propagation, controlled pollination, and progeny testing. These breeding activities are specifically designed to enhance the characteristics of the black wattle species (Sewpersad, 2004). Furthermore, rigorous field trials have to be conducted to evaluate the performance of new varieties across diverse environmental conditions before the new varieties can be made available to growers (Moreno Chan and Isik, 2019).

The evaluation of trees from the trials involves the collection of growth data, including measurements of tree height and diameter at breast height (DBH). Mean Annual Increment (MAI) is also recorded, and ongoing assessments are conducted to monitor growth performance (Beck *et al.,* 2007). In contrast to growth measurements, evaluating bark properties requires destructive or semi-destructive sampling, which involves removing portions of the bark from the tree. Additionally, the standard Society for Leather Technologists and Chemists (SLTC) methods that are commonly used to determine bark quality, are time-consuming, which limits the number of samples that can be analysed and hinders the accuracy and comprehensiveness of assessments (Gordon-Gray, 1953 and Avadianund Bridglall *et al*., 2025).

In a recent study on black wattle bark carried out by Avadianund Bridglall *et al*. (2025), the use of NIRS was found to be valuable in the assessment of bark quality parameters. The study allowed for 26 bark quality parameters to be successfully modelled (Chapter 3 of current thesis). NIRS has become widely popular due to its speed and cost-effectiveness, and its non-destructive and non-invasive nature. Its versatility allows it to be adapted to meet the specific needs of various industries. NIRS is particularly valuable because it can quantify chemical compounds using their unique light-absorbing properties (Ozaki, 2022). This technology is increasingly being adopted in forestry and agriculture worldwide for applications such as wood analysis, seed quality assessment, and pest and disease detection (Wang *et al*, 2022).

The aim of this study was to estimate bark quality in breeding trials using NIRS models. The specific objectives were as follows:

1. **Develop a predictive model for bark sample analysis:** develop a robust NIRS model to analyse bark samples collected from black wattle trees. The model would use South African Leather Technologists' Research Centre (SLTC) as the reference data to assess key chemical and physical properties. The model would help to streamline the plant breeding evaluation process by providing consistent, semi-destructive, and rapid assessments of bark quality of different parent plants and clones.

2. **Assess quality parameters of samples taken from across multiple trial sites:** These parameters would include Extractives, Tannin Content, Non-Tannins and bark extract colour (Lovibond Red and Lovibond Yellow).
3. **Investigate relationships between soil parameters and Wattle bark extract quality parameters:** analyse the relationship between site-specific soil conditions (e.g., soil pH, mineral content, texture) and the quality parameters of bark samples taken from these sites. By establishing correlations between these factors, the study aims to understand the interactions between genotype (tree genetics) and environment (site conditions). This insight may identify the best combinations of genetic lines and site conditions, enhancing the productivity and performance of black wattle varieties in plantations.

These objectives collectively support the improvement of breeding strategies, resource allocation, and site selection, to optimise the economic and ecological outcomes of black wattle cultivation.

6.3 Materials and methods

6.3.1 Description of the black wattle trials sampled

Two different field trial series were sampled in the study. Each of the trial series was established with different genetic material. These are described as follows:

6.3.1.1 Seed-source

The two seed source trials sampled were established in November 2015, one near Iswepe, Mpumalanga, and the other near Luneburg, KwaZulu-Natal. The trials were planted with bulk seed collected from six seed sources: PSO 10, PSO 11, PSO 14, PSO 16, Sheepmoor and Liff and were part of a frost tolerance study (Moreno Chan, 2019). It is important to note that the trees sampled in the block trials were a sample of the progeny from the different seed sources, not the seed sources themselves. In other words, the bark quality of the seed sources was estimated through the trees sampled in the block trials. The seed source block trials will be denoted simply as seed source trials for the rest of the chapter.

The seed sources are briefly described as follows: PSOs are production seed orchards that have supplied (PSOs 14, 16), or are currently supplying (PSOs 10-11),

black wattle seed to commercial nurseries. PSOs 10, 11 and 14 were established on mid-altitude (1120-1128 m asl) sites in KwaZulu-Natal with seeds of diverse genetic origin (Moreno Chan, 2019). PSO 16 was established on a mid-altitude (1178 m asl) site outside Piet Retief, Mpumalanga, with seed from superior trees selected in commercial stands in the Iswepe and Piet Retief areas (Iswepe/Piet Retief landrace). The seed sources of Sheepmoor and Liff were experimental bulk seedlots collected from two breeding trials. Sheepmoor was a block trial established in 1995 on a high-altitude (1458 m asl), cold site near Panbult, Mpumalanga with seed from two cold-hardy Australian provenances. Liff was a progeny trial established in 2002 at a moderately high-altitude site (1290 m asl) in the Karkloof, KwaZulu-Natal. This trial was established with seed from the same cold-hardy Australian provenances used in Sheepmoor, plus seed from wild trees growing at high altitude sites in the Drakensberg mountains. Further information on all the seed sources used in the current study was documented by Moreno Chan (2019).

The site characteristics and experimental design of the seed source trials are shown in Table 6.1 and Figure 6.1.

6.3.1.2 Clonal trials

The two clonal trials (CLT2-3) sampled in the current study were planted in September – November 2017 in the Paulpietersburg and Dalton areas, KwaZulu-Natal (Table 6.1). The site characteristics and experimental design of these trials are shown in Table 6.1 and Figure 6.2.

The clones used to establish the CLT2-3 trials were a small subset of selections cloned in 2012-2013 primarily to establish clonal seed orchards CSOs 1-3, which are currently supplying of improved seed to commercial nurseries. The clonal plants used in CLT2-3 were material left over and grown in 5 L bags at the ICFR nursery. In turn, the cloned selections were made in progeny trials established at sites in KwaZulu-Natal in 2002-2004, each of them being of different genetic origin and level of improvement (Moreno Chan and Isik, 2019). The trials collectively formed part of the ICFR's multiple population breeding strategy for black wattle (Dunlop et al. 2003).

It is important to indicate that none of the clones used in CLT2-3 trials has been, or is currently, deployed commercially. These are a subset of clones that were used to establish CSOs 1-3.

Table 6.1 Site characteristics and experimental design of trials sampled.

TRIAL	BT_CREYDT	BT_LUN2	CLT2	CLT3
Type of Trial	Seed source	Seed source	Clonal	Clonal
Farm, Location	Creydt, Iswepe (Mpumalanga)	Tamboekiesbult, Luneburg (KZN)	Schwarzwald, Paulpietersburg (KZN)	Harden Heights, Dalton (KZN)
Landowner	Norman Creydt	Hugo Niebuhr	Hugo Niebuhr	UCL
GPS Coordinates	26.913 S, 30.6091 E	27.3542 S, 30.6609 E	27.4445 S, 30.74653 E	29.244951 S, 30.652979 E
Altitude	1414 m	1167 m	1364 m	NA
Establishment Date	19/11/2015	12/11/2015	27/09/2017	24/11/2017
Spacing	3 x 1.5 m	3 x 1.5 m	3 x 1.5 m	3 x 1.5 m
No. seed sources/clones	6	6	15	7
Trial design	RCB, square plots of 36 trees, three replications	RCB, square plots of 36 trees, three replications	RCB, line plots of five trees, four replications	RCB, line plots of five trees, four replications
Total No. trees sampled	90	90	90	42
Age at bark sampling (years)	6.4	6.4	4.6	4.4

6.3.2 Bark Sampling Procedures

6.3.2.1 Tree sampling

Bark samples were taken in the same way at all four trials mentioned above. The trees that were sampled were healthy and of average growth. Suppressed trees and trees that exhibited any form of disease or stress were avoided. A 10 cm x 10 cm sample window of bark was taken at a height of 1.3 m using a stainless-steel cleaver. Stainless steel is recommended as it reduces the potential for iron contamination, which is known to affect colour (Slabbert, 1992). The bark samples were placed in plastic bags, vacuum-sealed, and placed in a cooler box with ice blocks to prevent degradation. The samples were transported to the ICFR laboratory on the same day of collection, weighed and freeze-dried to prevent degradation.

At the seed source trials, 15 trees per seed source were sampled across the three replications in each trial, i.e. five trees at each plot. Thus, a total of 90 trees were sampled in each of the seed source trials. Figure 6.1 below shows both the trial layout, as well as the trees sampled. The bark samples were collected in March - April 2022 (late summer - autumn), thus the trees were approximately 77 months (6.4 years) old at the time of sampling.

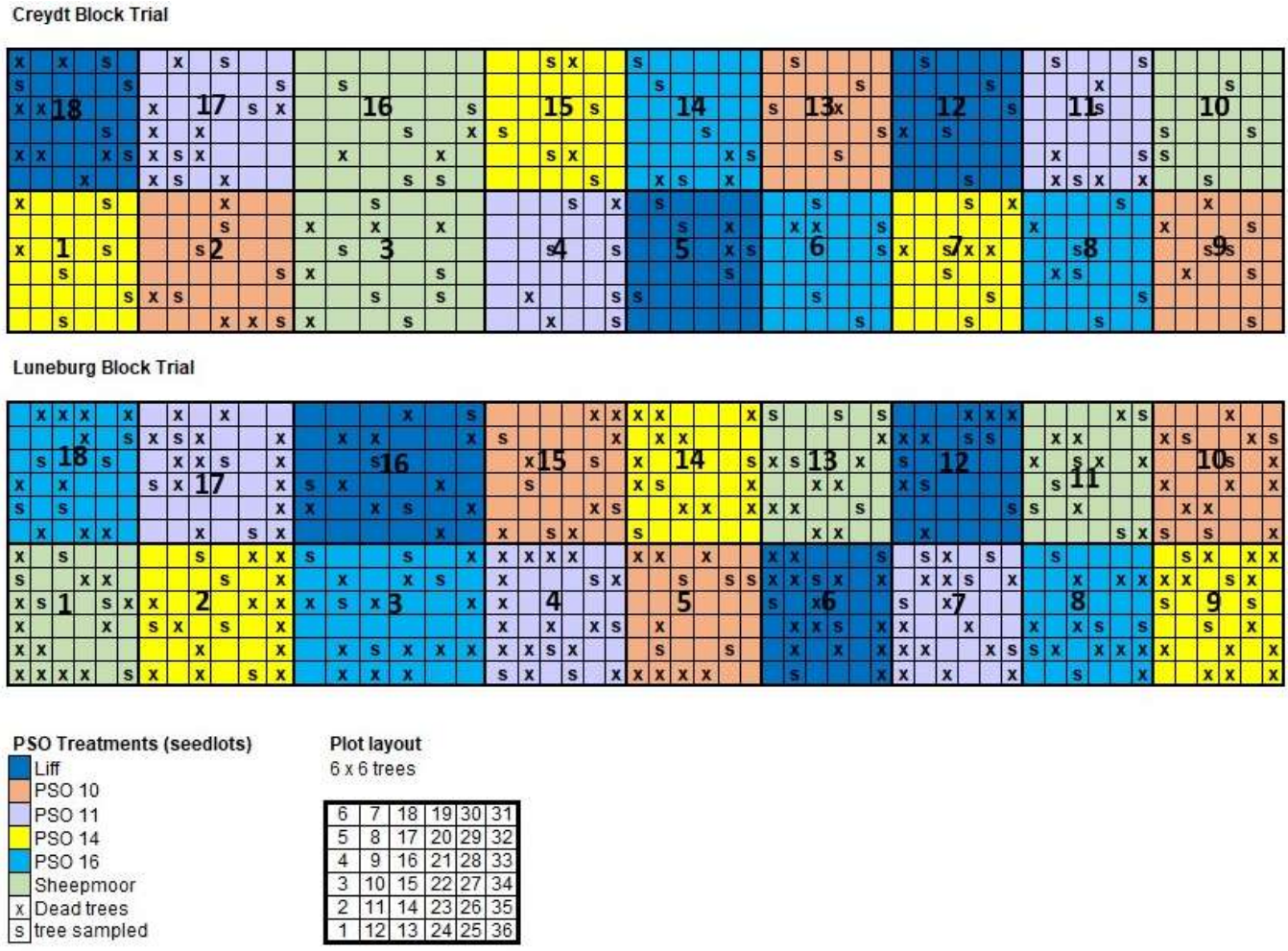


Figure 6.1 Seed source trial layout for Creydt and Luneburg sites, indicating the sampled trees and dead trees.

At the clonal trials, six trees per clone were sampled in each trial. More specifically, two trees per plot were sampled in three replications, with one replication not being sampled. At clonal trial CLT2 there were 15 clones, therefore a total of 90 trees were sampled. At clonal trial CLT3 there were only 7 clones, therefore a total of 42 trees were sampled. Figure 6.2 below shows both the trial layouts, and the trees sampled.

The bark samples were also collected in March-April 2022 (late summer - autumn). The trees at the CLT2 trial were approximately 55 months (4.6 years) old at the time of sampling. At the CLT3 trial, the trees were approximately 53 months (4.4 years) old at the time of sampling.

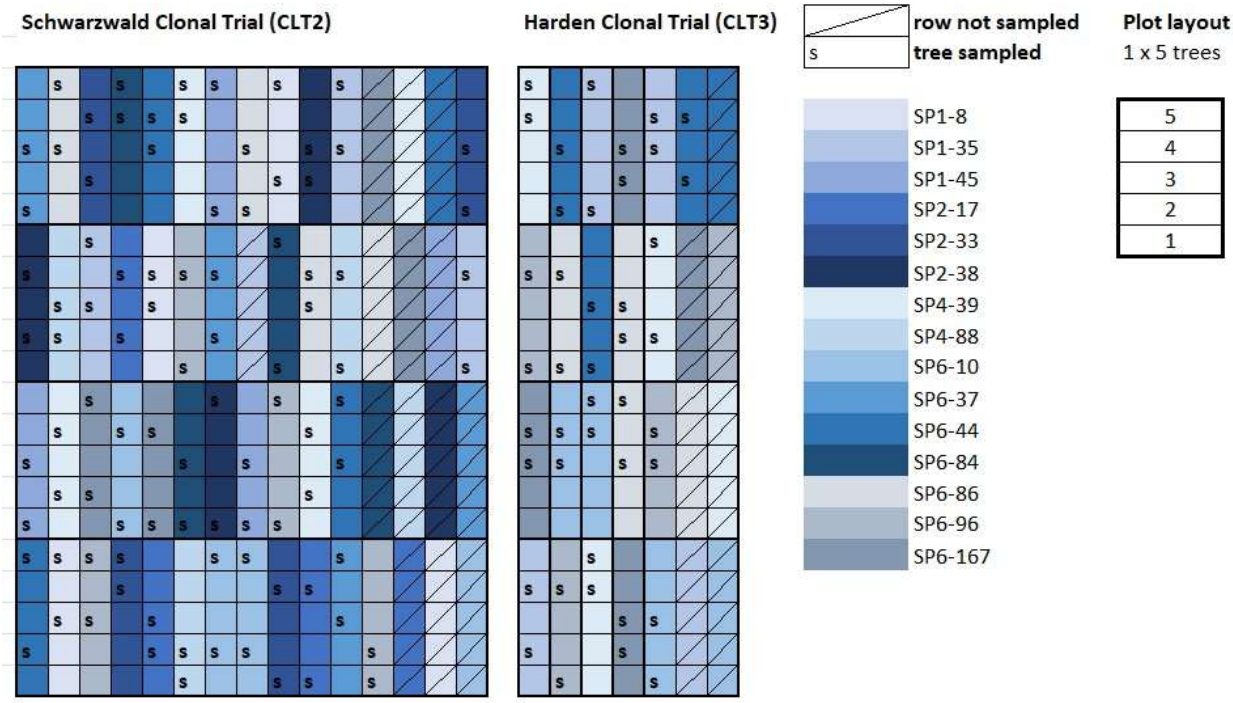


Figure 6.2 Clonal trial layout indicating trees sampled. The Schwarzwald trial included 15 clones, whereas the Harden Heights trial included only seven clones.

6.3.2.2 Bark sample moisture and milling

The bark samples were first weighed to determine their wet mass and then placed in a Virtis BT Pro Series freeze dryer (Lab 1st, USA) at -70 °C and 175 millitorr (mTorr) for 120 hours, or until a constant mass was reached. The final mass was recorded to determine the moisture content. Box and whisker plots were employed to compare differences for each variant in both the seed source and clonal trials grown at each site.

All freeze-dried bark samples were subsequently milled to a particle size of 0.5 mm using a ZM200 Retsch mill (Retsch, USA). The milled samples exhibited a moisture content of less than 1%. Samples were stored at -20°C to preserve integrity and prevent degradation prior to further analysis.

6.3.3 Bark Analysis

6.3.3.1 Reference Chemistry (SLTC) and NIRS model development

A combined NIRS model was developed using scans of material from all four trials sites. Approximately 20 % (62 freeze-dried) , milled bark samples were randomly selected (at least one sample per plot was selected) before being subjected to wet chemistry analysis.

6.3.3.1.1 Bark extraction and SLTC Analysis (Reference Chemistry)

The Double Autoclave Water method (DAW) was used to extract the contents of the freeze-dried, milled bark samples (Avadianund Bridglall *et al*, 2025). A 1:100 ratio of sample to distilled water by volume is used for this extraction process. The sample was extracted in two sequential autoclave cycles (under fixed temperature and pressure). The resultant extract was analysed using the SLTC methodology, i.e., extractives and tannin content analysis (Method SLT 2/3e), non-tannin content (Method SLT 2/3d), total soluble content (Method SLT 2/3c), total insoluble content (Method SLT 2/3f), and colour assessment (Method SLT 2/3g) (Leather Technologists Pocket Book, Leafe, 1999).

The different quality parameters were determined as follows:

1. **Extractives:** determined by drying the extract solution and recording the remaining solid mass.
2. **Non-Tannin Content:** the extract is treated with chrome-prepared animal hide powder, and the unbound material is dried and weighed.
3. **Tannin Content:** calculated using the formula below:

   % Tannin = % Extractives – (% Non-Tannin + % Insoluble).
4. **Colour (Red and Yellow):** colour is measured within 30 minutes of extraction using a 10 mm cell on a Lovibond Model E Tintometer after filtration through GF/C (Merck, Germany) pads. The colour is corrected, based on the extractives and tannin content, as per the formula below:

   Corrected Lovibond Colour of bark extracts = (Lovibond colour x aliquot of extract filtered x extractives % ÷ tannin % ÷ volume of extraction solution ÷ mass of dry bark)

Lovibond colour analysis does not use formal SI units (Gibson,1927), therefore in this work, it was reported as follows:

Lovibond Red = LRU
Lovibond Yellow = LYU

6.3.3.1.2 NIRS model development

A total of 312 freeze-dried, milled bark samples were scanned using a Bruker MPA FT-NIR spectrometer (Bruker, USA) at a controlled temperature of 20˚C. Each sample was scanned in triplicate to ensure measurement consistency. Additionally, the 62 samples that provided the wet chemistry reference data that were used to create the calibration and validation models, were also scanned in triplicate. This approach was employed to improve repeatability, minimise sampling error, enhance spectral data quality, strengthen the robustness of the chemometric model, and aid in the detection of anomalies (Kays *et al.,* 2000).

Data processing was conducted using the OPUS QUANT software (version 8.5, Bruker, Germany). Wet chemistry reference data were incorporated for each of the 62 reference samples scanned. Preprocessing was performed using vector normalisation and Partial Least Squares Regression as discussed by Avadianund Bridglall (2025). Among the methods evaluated, General B preprocessing proved to be the most versatile, as it applies enhanced smoothing and utilises multiplicative scatter correction. This is particularly effective for complex, overlapping samples (Miller, 2021).

To evaluate the performance of the calibration and validation models, the Coefficient of Determination (R^2), Root Mean Square Error of Prediction (RMSEP), and Ratio of Performance to Deviation (RPD) were recorded for each parameter (Extractives %, Tannin %, Non-Tannin %, Lovibond Red, and Lovibond Yellow). These statistical measures are essential in assessing how well the NIR model fits the reference data and its predictive accuracy. As a final step, the Ratio of Performance to Inter-Quartile Distance (RPIQ) was calculated to further assess model robustness. RPIQ, which is determined by dividing the interquartile range (IQR) of the reference values by the RMSEP, provides an additional indication of prediction performance, and is particularly valuable when the target data are non-normally distributed or contain outliers (Cen and He, 2007).

Once optimal models were developed for each parameter, using the validation data, they were applied to an independent test set to assess predictive performance. A set of twenty samples, not previously used during model development, was randomly selected. These samples underwent independent wet chemistry analysis to generate reference values for comparison with the NIRS-predicted results.

Analysis was conducted using the Quant Analysis module of the OPUS software package (version 8.5, Bruker, Germany), where the sample spectra were processed using the established prediction models for each parameter. Statistical comparisons between the predicted and reference values were performed to assess model accuracy, with performance evaluated in terms of correlation and agreement with the true values of the independent test set. Lin's Concordance Correlation Coefficient (Lin's CCC) was calculated for each using statstodo.com (Accessed April 2025).

6.3.4 Soil Samples Collection and Analysis

Three sets of soil samples were collected at each of the trials. The soil sample points were located near the two extremes and the centre of each of the trials. These consisted of soil taken at 0 - 5cm (surface soil), 5 - 20 cm, 20 – 40 cm, 40 – 50 cm, 50 – 80 cm, and 80 – 100 cm (Figure 6.3). Approximately 250 g per sub-sample was collected using a Dutch auger.

Figure 6.3 Illustration of soil samples taken at the different depths. (Photo: Bridglall, 2022).

The samples were stored in plastic bags and transported in a cooler box to the ICFR. The samples were air-dried for 2–3 weeks, milled, and sieved (2.0 mm and 0.5 mm) before analysis. The 2.0 mm was used for all analysis except Total Carbon, Nitrogen

and Sulphur, that utilised the 0.5 mm sieved soil samples. Analyses were performed at the ICFR laboratory following the standard methods, as adapted from Donkin *et al.* (1993).

For each site, the three soil samples were analysed individually for the parameters listed below:

- **Moisture Factor (mf):** Calculated as the ratio of air-dried to oven-dried mass (dried at 105°C for 24 hours).
- **pH (KCl and H_2O):** Measured using sieved, air-dried soil mixed with either 1M KCl solution or deionized water and left to equilibrate overnight. Measurements were performed with a calibrated pH meter.
- **Exchangeable Acidity:** Determined via titration with 0.01M NaOH using phenolphthalein as an indicator. Results were expressed in cmolc kg^{-1} soil.
- **Exchangeable Cations** (Calcium (Ca), Magnesium (Mg), Potassium (K), and Sodium (Na)): Extracted with 1M ammonium acetate, filtered, and analysed using an Agilent MP-AES. Results were converted to oven-dried mass and reported in cmolc kg^{-1} soil.
- **Micronutrients** (Copper (Cu), Iron (Fe), Manganese (Mn), and Zinc (Zn))**:** Quantified using an Agilent MP-AES, with results reported in mg kg^{-1}.
- **Bray II Phosphorus:** Assessed to measure total, organic, and available phosphorus using the Bray and Kurtz (1945) method.
- **Total Carbon, Nitrogen, and Sulphur (CNS):** Analysed with a Leco Trumac CNS analyser, with results plotted graphically.

The different depths and individual samples were combined, and the data were compared using Bar charts created in Excel (2025, version 2505 (Build 18827.20150)). The two soil quality parameters for the seed source trials were compared against each other, and the data for the clonal trials were compared against each other.

6.3.5 Data Analysis

Fisher's Least Significant Difference (LSD) plots were used to compare the quality parameters of each seed source/clone at each site. Principal Component Analysis (PCA) was conducted to evaluate the relationships between each variety and its

associated quality traits, as well as site-specific soil variables. The PCA plots were refined to display only statistically significant associations, with non-contributing variables excluded for clarity. All statistical analyses were performed using R Studio (Cran, version 2025.05.1+513). R packages used for the LSD plots were agricolae and ggplot2, for the PCA plots were stats, factoextra, ggplot2, FactoMineR, and ggbiplot.

6.4 Results

6.4.1 Moisture content

The moisture content data for the different seed source and clonal trials are shown in the box and whisker plots in Figures 6.4 (Seed source Trials) and Figure 6.5 (Clonal Trials) below.

In the seed source trials, Luneburg had higher moisture content than Creydt for some seed sources. As expected, there was variation within seed sources in both sites (Figure 6.4), but this could be related to experimental sampling.

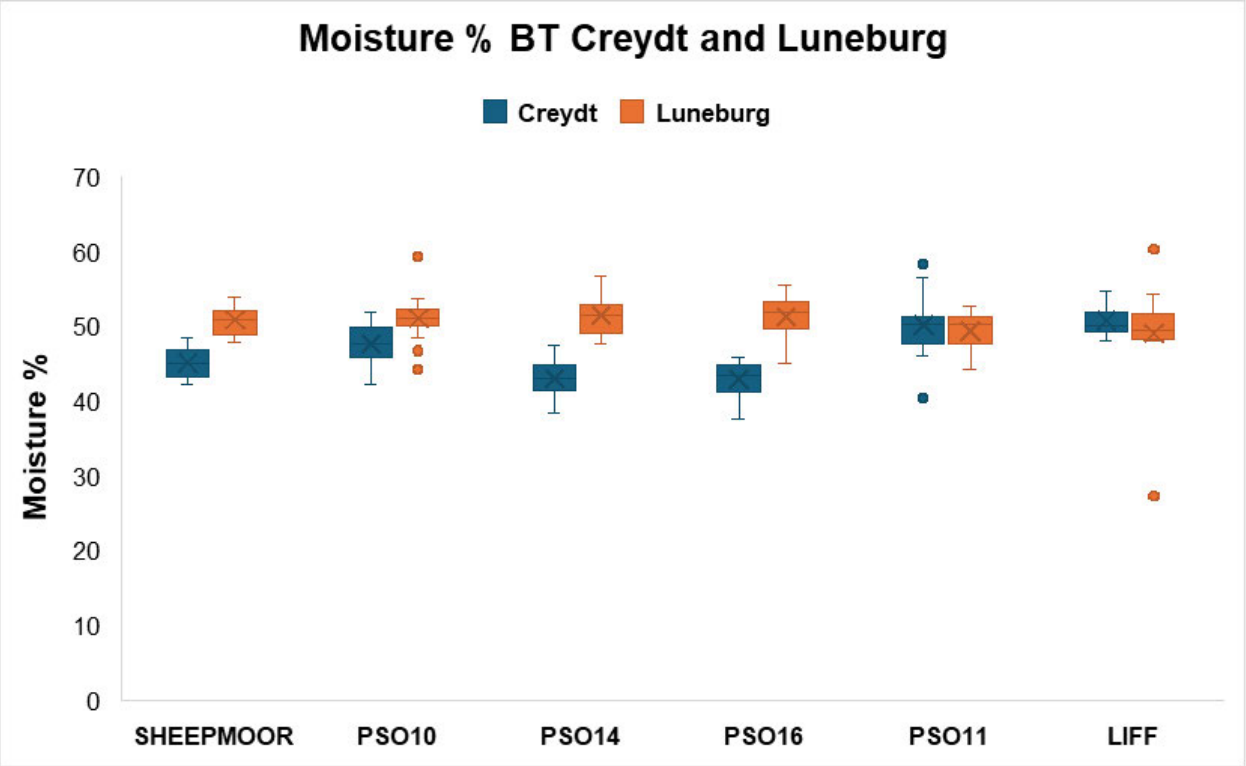


Figure 6.4 Plot of Moisture % for seed source trials at Creydt and Luneburg. The center line in the box is the median, the dots are indicative of outliers, and the size of the box shows the standard deviation in measurements for each variant.

The moisture % for the Harden Heights samples was higher than for the Schwarzwald samples. The Harden Heights varieties also had more variation between trees of the same variety. Harden Heights only had seven clone varieties, whereas Schwarzwald has fifteen, as seen in Figure 6.5 below.

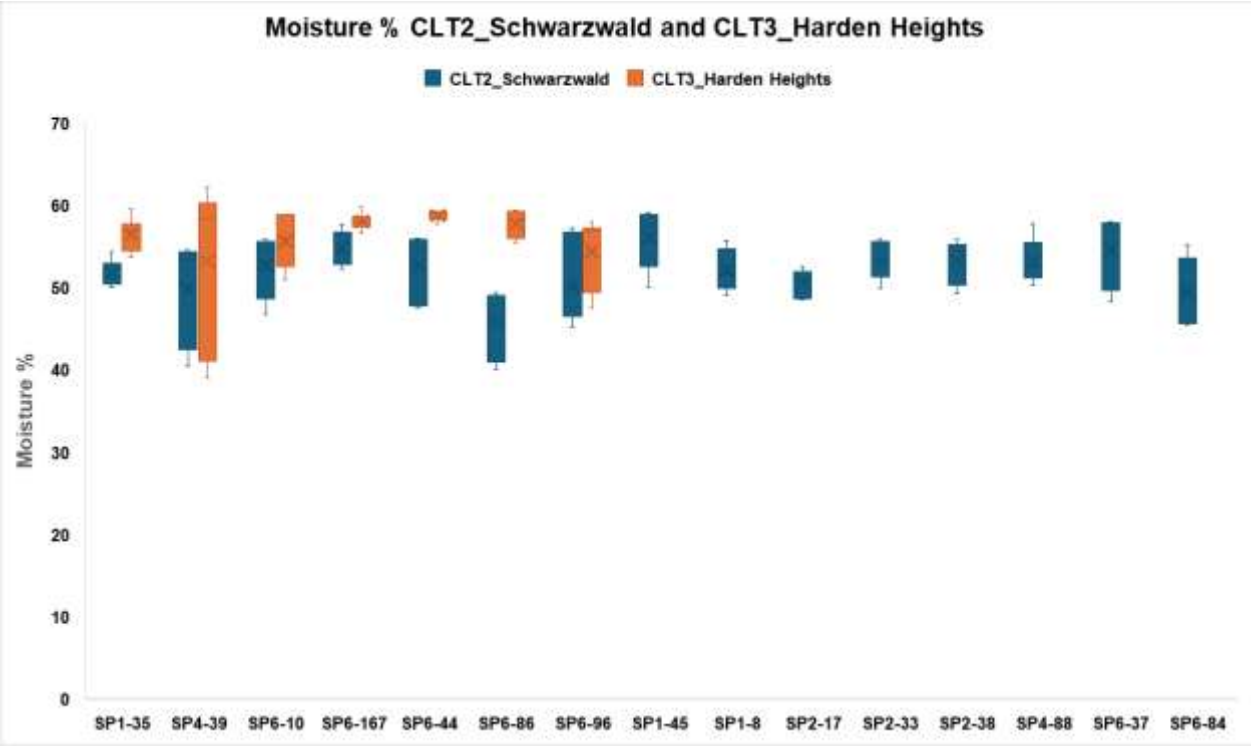


Figure 6.5 Box and whisker plot of Moisture % for Clonal Trials CLT2_ Schwarzwald and CLT3_Harden Heights. The line on the bar shows the standard deviation, the center line in the box is the mean, the dots are indicative of outliers, and the size of the box shows the variation in measurements for each variant.

6.4.2 NIRS Models

6.4.2.1 Calibration and Validation data

A combined NIRS calibration and validation model was created using the scanned spectra obtained from both the seed source and clonal trials. The calibration and validation data for the various bark quality parameters are presented in Table 6.2.

The predictive performance of the models was good, especially for tannin (R^2 = 89.8 %, RPD = 3.12, and RPIQ = 12.2), indicating excellent accuracy for quantitative analysis. The models for Extractives, Non-Tannin %, and Lovibond colour parameters also show good reliability, with RPIQ values above 4, supporting their

use for semi-quantitative applications. Overall, the models are robust and suitable for routine, semi-destructive bark quality assessment.

Table 6.2 NIRS Calibration and Validation data of bark quality parameters for Seed source and Clonal Trials.

Parameter	Unit	Range (Min)	Range (Max)	Spectral Range	Calibration		Validation		RMSEP	IQR	RPIQ
					R^2	RPD	R^2	RPD			
Extractives	%	27.3	39.0	4736.6 – 3594.8	83.0	2.43	79.5	1.43	2.63	11.7	4.5
Tannin	%	16.2	25.8	7506.0 – 4242.8	90.5	3.36	89.8	3.12	0.79	9.6	12.2
Non-Tannin	%	7.8	13.4	13247.1-11563.7 7012.3 – 4728.8	83.7	1.33	76.8	1.07	1.19	5.6	4.7
Manual Lovibond Red	LRU	0.5	1.2	7506.0 – 6094.3	91.1	3.34	87.6	2.84	0.08	0.7	8.8
Manual Lovibond Yellow	LYU	0.7	2.3	10429.7 – 9288.0 8154 – 5870.6	76.9	1.93	73.2	1.56	0.25	1.6	6.4

6.4.2.2 Independent Test Sets

The correlations between predicted values (Validation data model) and the results from the independent test samples (wet chemistry -SLTC analysis) were evaluated using twenty samples. For each parameter, the R^2 and Lins CCC were calculated. The corresponding values are presented in Table 6.3.

The independent validation results confirm that the developed NIRS models offer excellent predictive performance for key bark quality parameters. The combination of high R^2 values, acceptable levels of concordance as indicated by Lin's CCC, and high prediction accuracy demonstrate that NIRS can serve as a reliable alternative to traditional SLTC wet chemistry methods for the assessment of black wattle bark samples for the quality parameters.

Table 6.3 Independent test set correlation for Seed source and Clonal Trial bark quality parameters**.**

Parameter	SLTC		NIRS		R^2	Lin's CCC	Accuracy
	Mean	Standard Deviation	Mean	Standard Deviation			
Extractives %	29.71	1.68	29.27	1.95	0.9976	0.6670	0.9616
Tannin %	19.08	1.22	19.34	1.41	0.9978	0.7418	0.9728
Non-Tannin %	8.55	0.64	8.66	0.77	0.9956	0.6368	0.9680
Lovibond Red	0.81	0.12	0.81	0.14	0.9861	0.7490	0.9837
Lovibond Yellow	1.74	0.23	1.83	0.26	0.9923	0.7216	0.9278

6.4.3 Soil properties

Figure 6.6 (A–D) and Figure 6.7 (A-D) present bar graphs illustrating the combined soil analysis results across all sampling depths and replicates for the Luneburg and Creydt sites. The data has been combined to provide a comparative overview of the soil conditions at each location, highlighting any major differences in soil chemical and physical properties. Each sub-figure (A–D) focuses on a distinct group of soil parameters.

The plots indicate that most soil quality parameters were relatively consistent between the two sites. However, notable differences were observed in the levels of macronutrients C and P, and the micronutrients Mn and Fe, which was observed to be higher at Creydt. Additionally, the soil texture varied between the sites, with Creydt exhibiting a sandier profile, while Luneburg being characterised by a higher clay content.

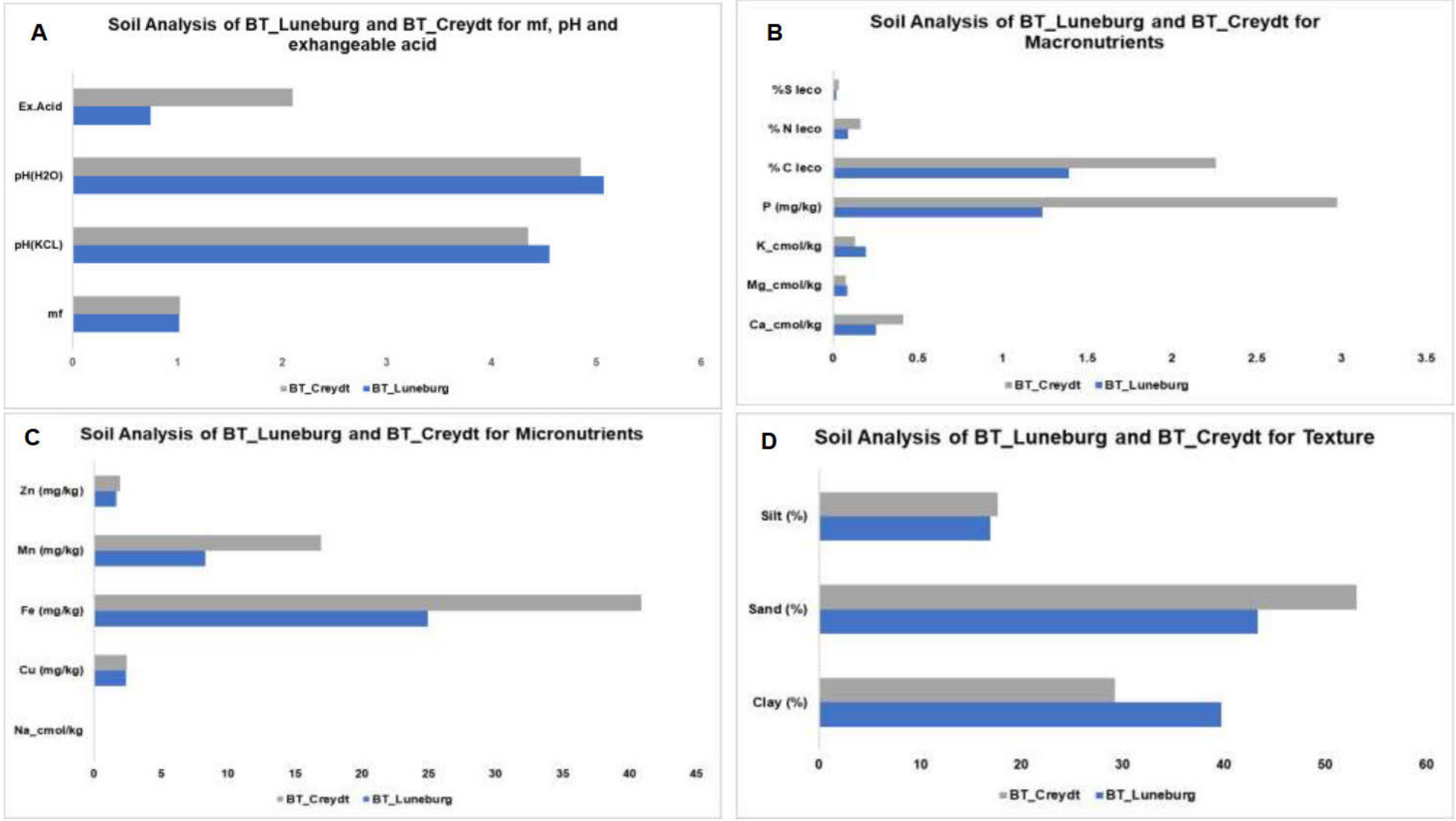


Figure 6.6 Bar plots for Seed source Trials at Creydt and Luneburg for (A) moisture factor (mf), pH, and exchangeable acid, (B) soil macronutrients (S, N, C, P, K, Ca, and Mg), (C) soil micronutrients (Zn, Mn, Fe, Cu, and Na), and (D) soil texture.

The bar plots in Figure 6.7 show differences in soil parameters between the Harden Heights and Schwartzwald sites. Harden Heights was characterised by higher levels of C, K, and Mn, and exhibited a predominantly clay-based soil texture. In contrast, Schwarzwald showed higher concentrations of P and Fe, and was associated with a sandier soil profile.

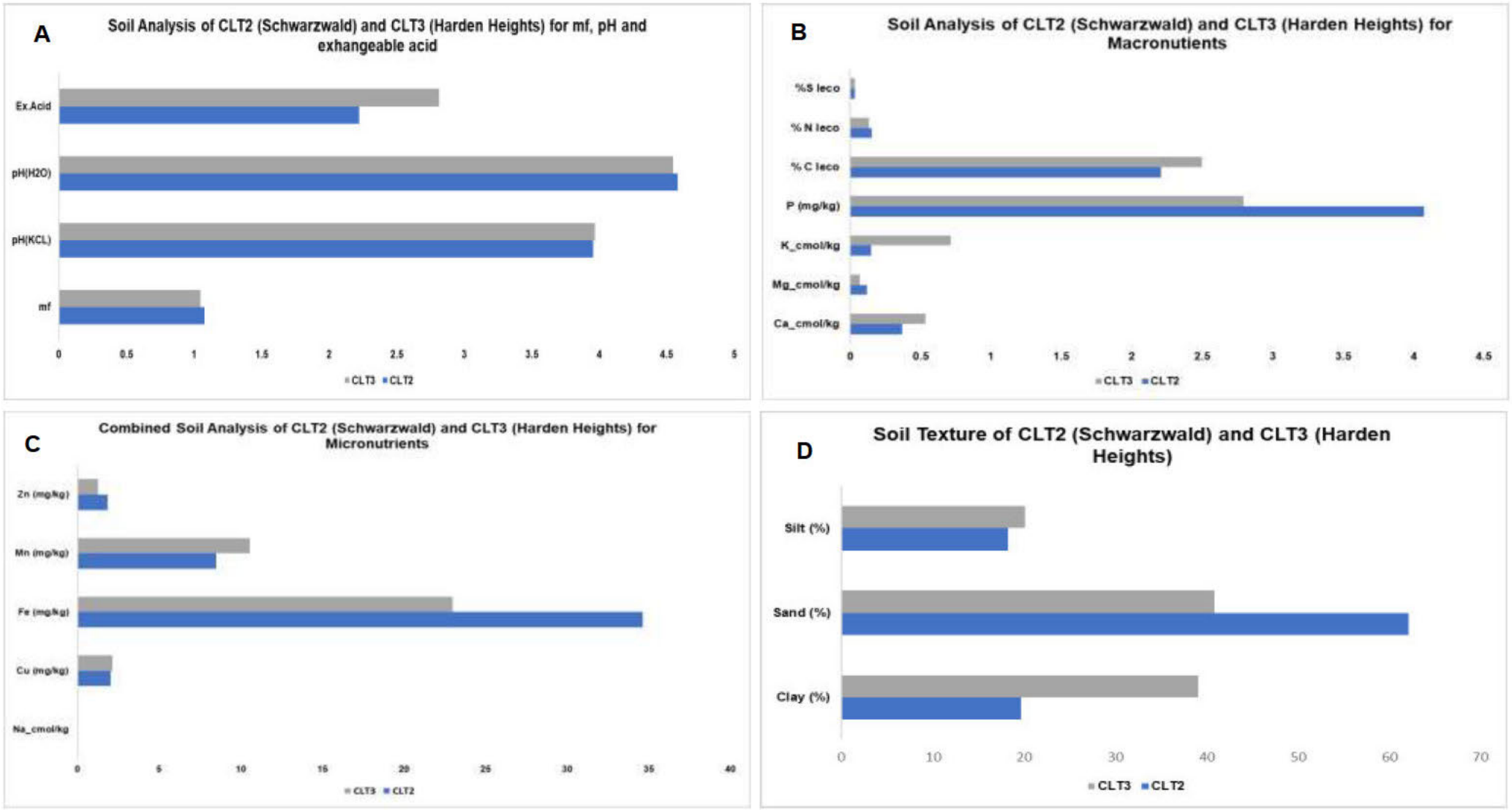


Figure 6.7 Bar plots for Clonal Trials at CLT2 (Schwarzwald) and CLT3 (Harden Heights) for (A) moisture factor (mf), pH, and exchangeable acid, (B) soil macronutrients (S, N, C, P, K, Ca, and Mg), (C) soil micronutrients (Zn, Mn, Fe, Cu, and Na), and (D) soil texture.

6.4.4 Data Analysis

6.4.4.1 Seed source trials

The LSD plots created for the different seed sources in both Creydt and Luneburg sites are shown below in Figure 6.8 (A-E)

The LSD plots show that samples from Creydt had higher values for the bark quality parameters, especially for Extractives %, Tannin %, and Non-Tannin %. Samples from Liff, PSO11, PSO14 and PSO16 showed similar tendencies towards Tannin % and Lovibond colour, whereas samples from PSO10 and Sheepmoor showed no significant differences to each other. Samples from Luneburg showed minimal variation across all bark quality parameters across all seed sources, except for Lovibond Red colour where samples from PSO16 were darker than all other seed sources.

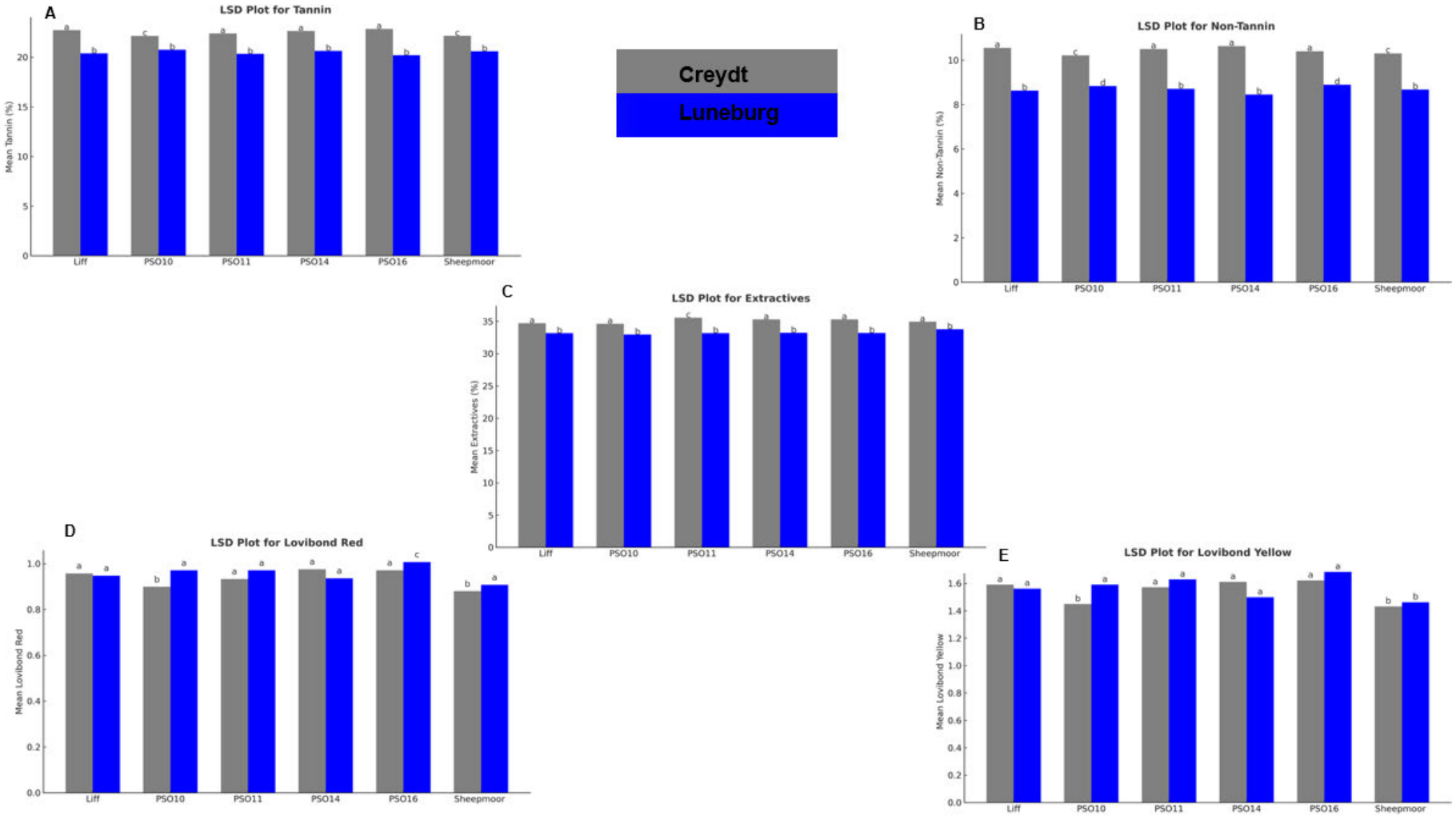


Figure 6.8 LSD plots comparing Bark Quality Parameters for (A) Tannins, (B) Non-Tannin, (C) Extractives, (D) Lovibond Red, and (E) Lovibond Yellow of Seed source Trials carried out at Creydt and Luneburg. Different letters mean differences were significant (P = 0.05) across both trails .

6.4.4.2 Clonal Trials

The LSD plots created for the different varieties in both the Clonal Trials for Schwarzwald and Harden Heights are shown below in Figure 6.9 (A-E)

The samples from clonal varieties at Schwarzwald (CLT2) showed much more variation in bark quality in comparison to samples taken from the same varieties at Harden Heights (CLT3). The Lovibond colour of samples from Harden Heights (CLT3) were lower than samples taken from the same varieties at Schwarzwald (CLT2), even though bark samples from Schwarzwald had higher Tannin % and Extractives %.

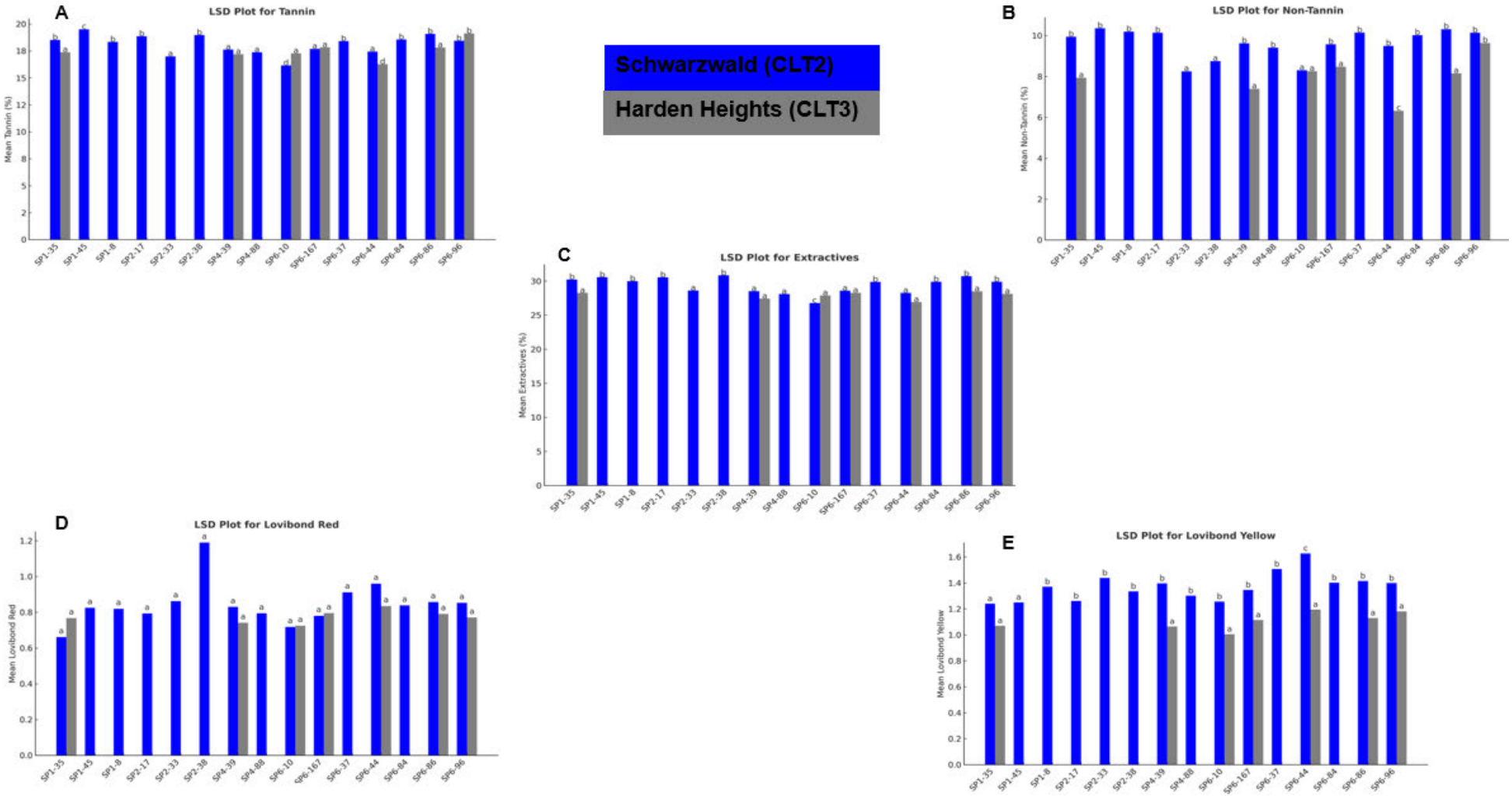


Figure 6.9 LSD plots comparing Bark Quality Parameters for (A) Tannins, (B) Non-Tannin, (C) Extractives, (D) Lovibond Red, and (E) Lovibond Yellow of bark samples taken from clonal trials carried out at Schwarzwald (CLT2) and Harden Heights (CLT3). Different letters mean differences were significant (P = 0.05) across both trails .

6.4.4.3 Site by genotype interactions

The site- by-genotype interactions were analysed using PCA biplots, comparing the different varieties from the Seed source trials and Clonal trials to the bark quality and soil quality parameters of the respective sites from which they were sampled. The PCA biplots are illustrated below in Figure 6.10, Figure 6.11 Figure 6.12, and Figure 6.13.

Figure 6.10 shows that the bark quality parameters (Extractives, Tannins, Lovibond red, Lovibond yellow, and Non-Tannins) are strongly correlated with one another. These variables are negatively associated with Sand %, while exhibiting weaker or variable associations with the remaining soil parameters including Fe.

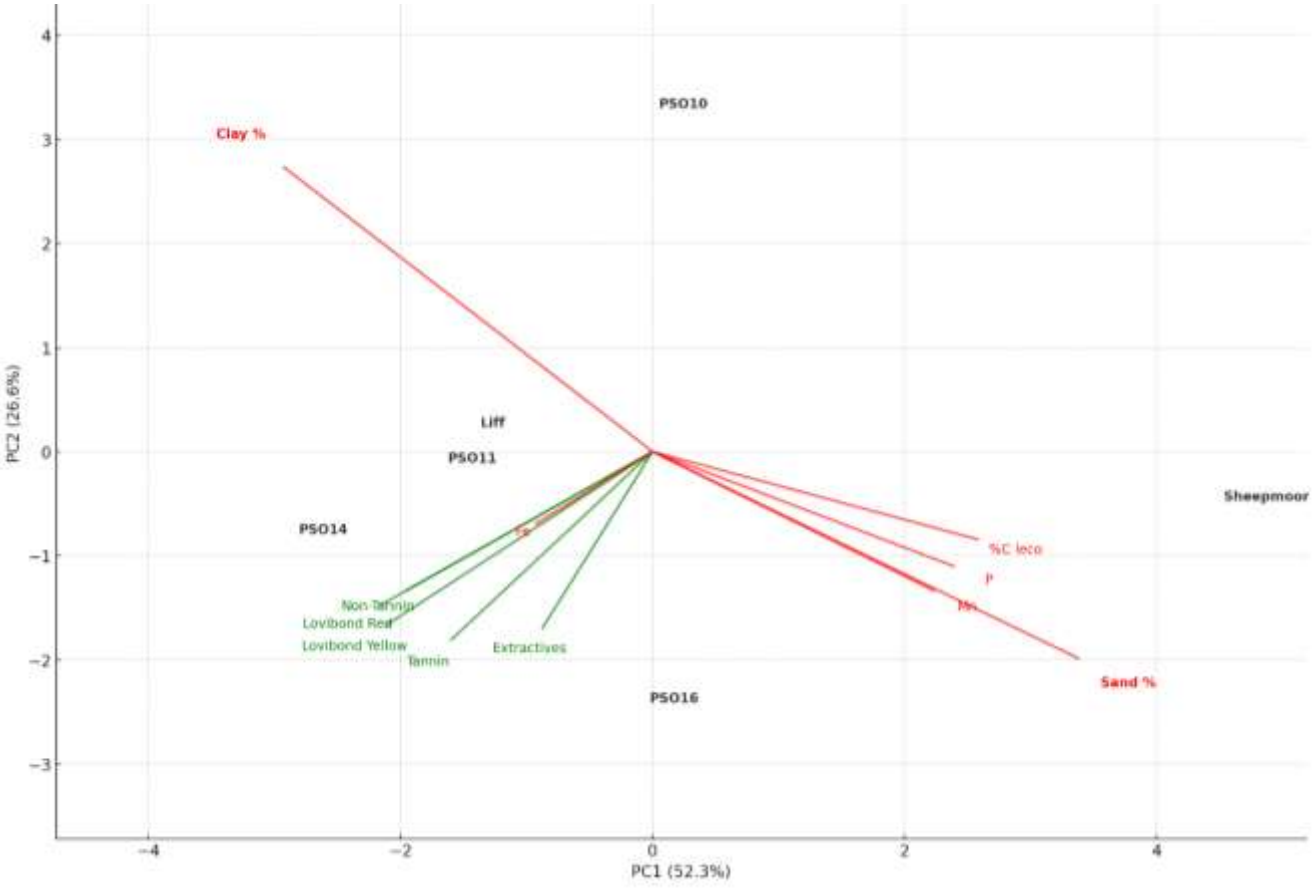


Figure 6.10 PCA biplot of the Seed source trial at Creydt showing the response of the different wattle seed sources (PSO10, PSO11, PSO14, PSO16, Liff and Sheepmoor) in response to bark quality parameters (Extractives %, Tannin %, Non-Tannin %, Lovibond Red, and Lovibond Yellow), and soil quality parameters (Macronutrients (P and C), Micronutrients (Fe Mn), and Soil texture (Clay % and Sand %).

Figure 6.11 shows the PCA biplot for the Luneburg site, depicting the multivariate relationships between soil characteristics and bark quality parameters as represented by the vector orientation and length. P is positively associated with colour and negatively with tannin, while extractives correlate positively with clay and negatively with Mn, Sand, and Fe. A contrasting site pattern is also evident between Creydt (Figure 6.10).

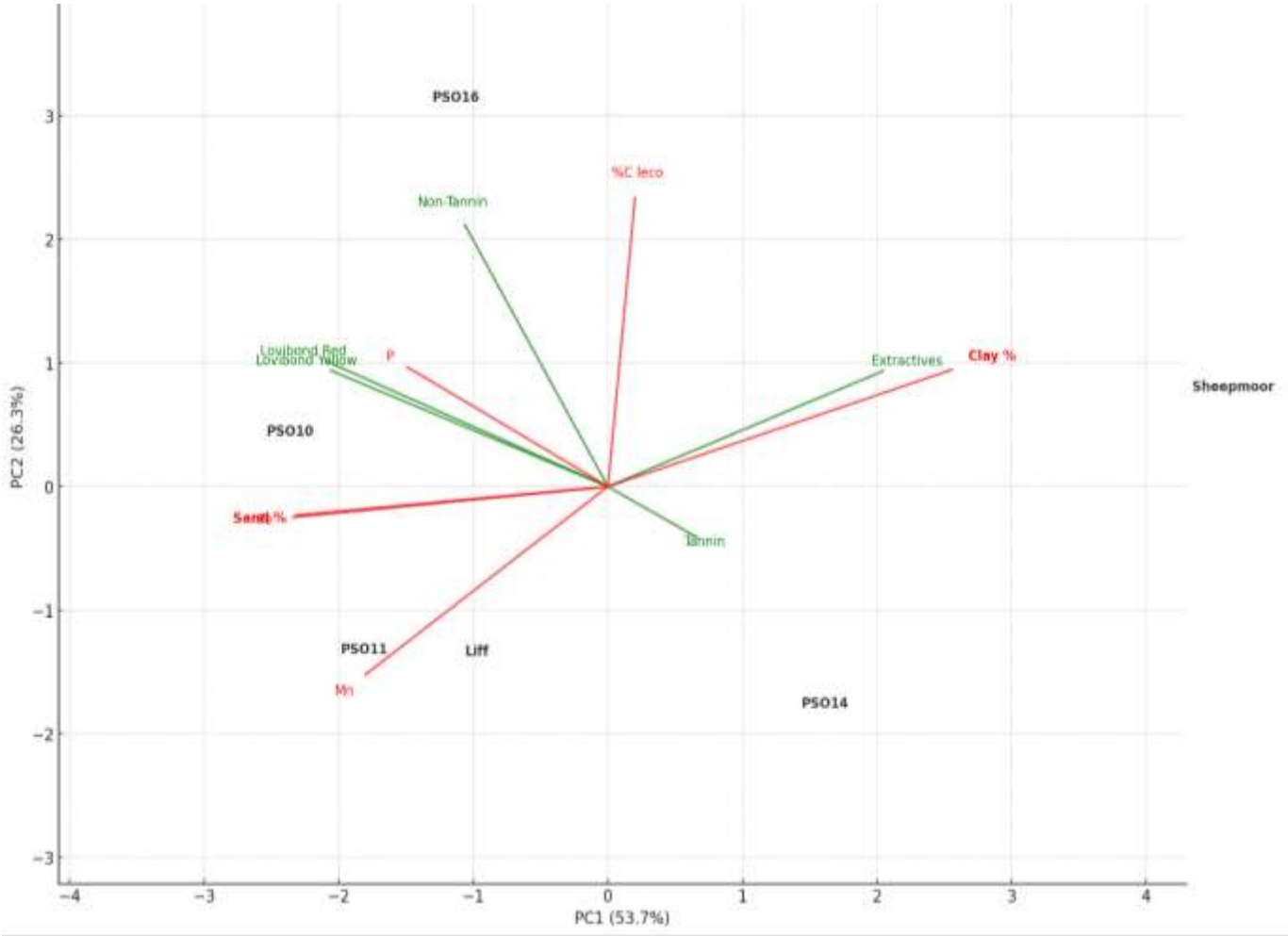


Figure 6.11 PCA biplot of the trial at Luneburg, showing the response of the different wattle seed sources (PSO10, PSO11, PSO14, PSO16, Liff and Sheepmoor) in response to bark quality parameters (Extractives %, Tannin %, Non-Tannin %, Lovibond Red, and Lovibond Yellow), and soil quality parameters (Macronutrients (P and C), Micronutrients (Fe Mn), and Soil texture (Clay % and Sand %).

The PCA biplot, Figure 6.12, for Schwarzwald (CLT2) , indicates that clay is not associated with extractives or tannin and shows a weak positive relationship with

colour parameters. Sand, % C, P, and Mn are negatively associated with colour while exhibiting little to no relationship with Non-Tannins.

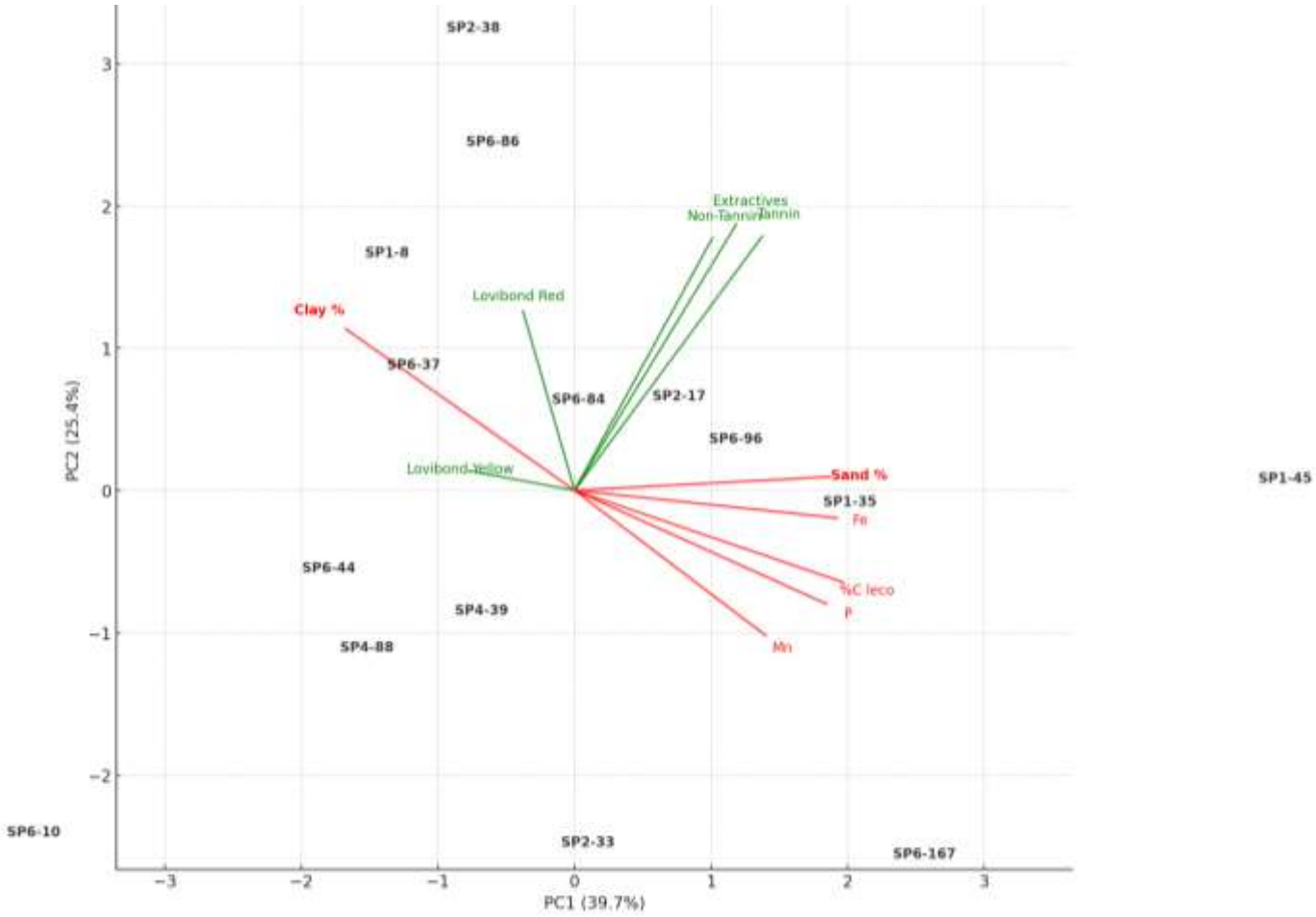


Figure 6.12 PCA biplot of the clonal trial at Schwarzwald (CLT2) showing the response of the different wattle varieties (SP1-8, SP1-35, SP1-45, SP2-17, SP2-33, SP2-38, SP4-39, SP4-88,SP6-10, SP6-37, SP6-44, SP6-84, SP6-86, SP6-96, and SP6-167) and in response to bark quality parameters (extractives %, tannin %, non-tannin %, Lovibond red, and Lovibond yellow), and soil quality parameters (Macronutrients (P and C), Micronutrients (Fe and Mn), and Soil texture (Clay % and Sand %).

The Harden Heights (CLT3) PCA biplot (Figure 6.13) shows strong relationships within the bark quality parameters, and moderate to weaker relationships between bark quality and soil parameters .

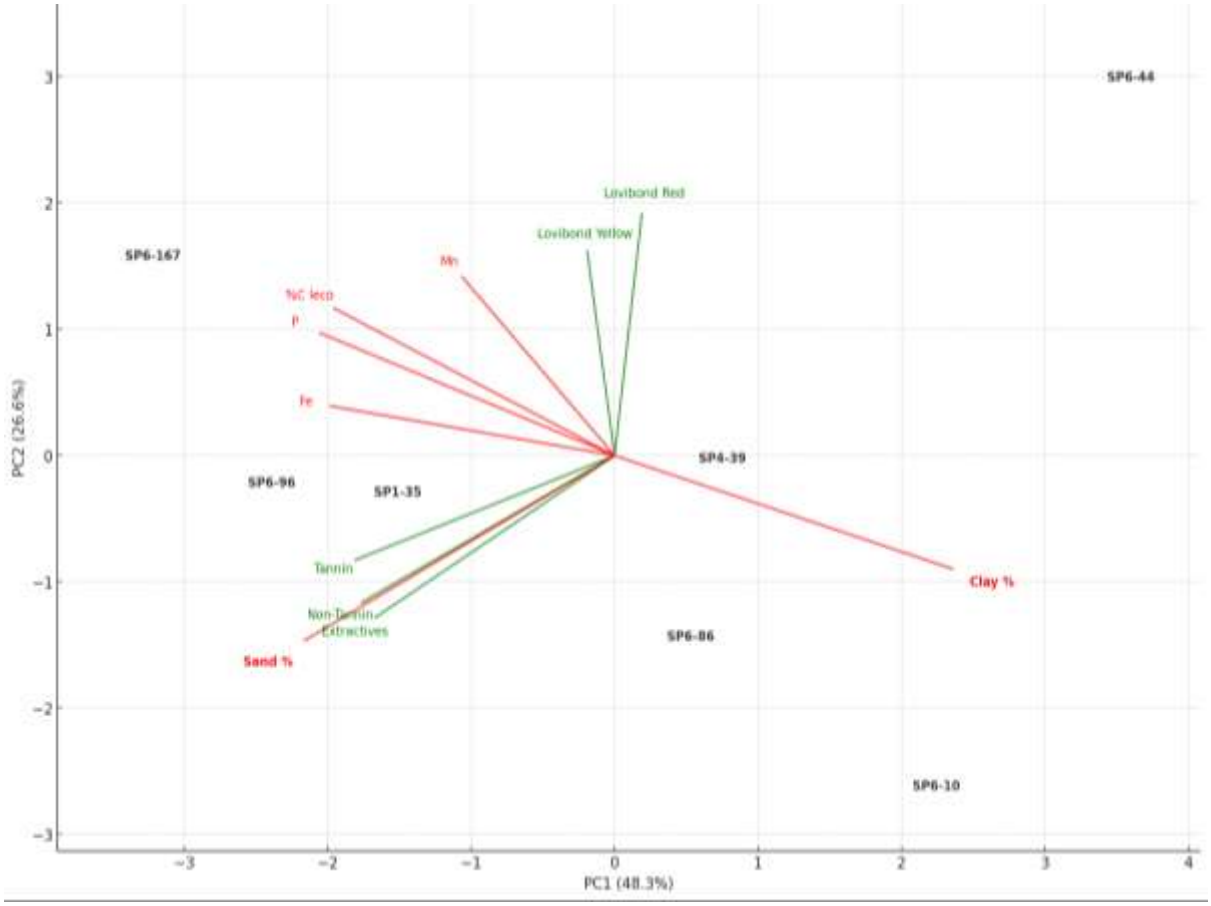


Figure 6.13 PCA biplot of Clonal Trial at Harden Heights (CLT3) showing the response of the different wattle varieties (SP1-35, SP4-39, SP6-10, SP6-44, SP6-86, SP6-96, and SP6-167) in response to bark quality parameters (extractives %, tannin %, non-tannin %, Lovibond red, and Lovibond yellow), and soil quality parameters (Macronutrients (P and C), Micronutrients (Fe and Mn), and Soil texture (Clay % and Sand %).

6.5 Discussion

This study was developed in response to ongoing work carried out at the ICFR, prompted by a technical report that showed that wattle extract quality is impacted by various environmental factors (Burgdorf and Germishuizen, 2021). Some of the mitigation strategies that were suggested included site-species matching, breeding for drought tolerance and ensuring moisture levels are maintained in wattle bark. However, due to large sample volumes being required for this research, the SLTC method of analysis is not feasible because it requires large sample volumes, is costly, and is slow (Avadianund Bridglall *et al.,* 2025).

The advances of NIRS have provided forestry and the agricultural industry with a powerful, non-destructive analytical tool for the rapid assessment of chemical and physical properties in plant materials (Wang *et al.,* 2002). The use of NIRS for various breeding strategies has been investigated since the early 1980's (Walker and Panozzo, 2023). This study focused on using NIRS to rapidly and accurately predict bark quality parameters of two different seed source trials and two clonal trials. The site-by-genotype interactions were also investigated.

Six-year-old trees were selected for this study to evaluate the feasibility of using NIRS for early-stage screening of bark quality traits in black wattle. While commercial harvesting typically occurs at ten years of age to maximise tannin yield and bark volume (Dunlop, 1998), this study aimed to determine whether earlier assessments could provide meaningful insights. Establishing the capability of NIRS to accurately predict quality traits in younger trees may support the development of more responsive breeding and site selection strategies (Dunlop *et al.*, 2005). It is recommended that future studies incorporate repeated assessments at two-year intervals to track bark quality parameters over time, enhancing model accuracy and enabling more precise selection of superior genetic material.

The moisture content was first determined in all samples taken by freeze-drying of the bark. Freeze-drying is less destructive than oven drying (Jain *et al.,* 2016) and does not destroy the chemical structures of the bark, unlike oven-drying. The plots in Figures 6.4 and 6.5 illustrate the variability in moisture content between sites and among wattle varieties. The Luneburg site showed higher average moisture values with greater variability across block trial varieties than the site at Creydt, which exhibited tighter clustering and lower mean. This may be attributed to Luneburg's higher clay content, as clay soils tend to retain more water than sandy soils, such as those at Creydt (Figure 6.6 (D)) (Brady & Weil, 2008). Similarly, the Harden Heights (CLT3) clonal trial displayed greater average moisture and variability than the Schwarzwald (CLT2) trial. Higher moisture of the bark is also associated with lower extractives in the bark, and this is seen in Figures 6.8 (C) and Figure 6.9 (C) where the Luneburg block trial varieties and the Harden Heights clonal trial varieties have lower extractives in relationship to the other sites they are compared to.

Freeze-drying of the bark also allowed for easy milling and scanning of the bark with NIRS. The freeze-dried, milled bark contained less than 1 % moisture, preventing the moisture from saturating the NIRS detector and reducing model accuracy (Cozzolino, 2009). The calibration and validation models for the developed NIRS showed good predictive capacity for bark quality parameters. Tannins % and Lovibond Red are two key quality traits routinely analysed in black wattle bark (Avadianund Bridglall *et al.,* 2025). The R^2 values obtained for Tannin %, and Lovibond Red were an average of 90 %, with low RMSEP and high RPIQ, which indicated the excellent predicative nature of the models. The R^2 values for Extractives, Non-Tannins and Lovibond Yellow were slightly lower but also produced values that were good, and which could be used for semi-quantitative prediction of the bark extract parameters (Williams & Norris, 2001).

The validation of the NIRS models using an independent test set (Table 6.3) further confirmed the robustness of the model. High R^2 values across all parameters (>0.90) and strong Lin's Concordance Correlation Coefficients (CCC values ranging from 0.6368 to 0.7490) demonstrated good linearity and agreement with SLTC wet chemistry methods. Accuracy values close to 1.0 confirmed the reliability of NIRS predictions (Lawrence and Lin, 1989). These results support the model's robustness and accuracy in predicting bark quality parameters across both the seed source trials and clonal trials because they align with findings in other forestry-based NIRS studies (Schimleck *et al.,* 2003).

Black wattle timber and bark yield are both impacted by soil quality. Black wattle performs well in deep, moist, fertile soils with moderate pH levels (Schonau, 1970). Poor soils have been linked to increased gummosis and reduced bark quality (Booth, 1997). Soil analysis bar plots (Figures 6.6 and 6.7) revealed differences in soil properties that may influence bark quality. In the seed source trials, the Creydt soil showed elevated levels of macronutrients (C and P) and micronutrients (Mn, Fe), along with a sandier texture. These soil parameters are known to affect wattle bark quality (Avadianund Bridglall *et al*, 2025). The clonal trials, soils at the Harden Heights site had higher C, K, and Mn levels and a clay texture, while the soils at the Luneburg site had elevated P and Fe with sandy soils. These site-specific differences would contribute to genotype-by-environment interactions, impacting on

trait expression such as tannin synthesis and moisture retention (Olivoto *et al.,* 2017).

The LSD plots (Figure 6.8 A–E) compare the bark quality parameters across block trial varieties at Creydt and Luneburg. The plots for Luneburg showed consistency across all bark quality parameters and varieties. The plots for Creydt showed more variation in data, but also showed higher Extractives %, Tannin %, and a lower Lovibond colour than for Luneburg. This was also consistent with the initial sample data, where the samples from Creydt had lower bark moisture, hence more extractives and tannin. It is also worth noting that samples from Creydt had a higher Non-Tannin % in comparison to samples from Luneburg, which can be attributed to various factors such genetic variation especially in younger trees, environmental influence, and less tannin production due to reduced site stress (Dunlop, 1998 and Hagerman, 2002). The seeds of trees from Liff had the highest Tannin %, although trees from Sheepmoor and PSO1O seed had the lowest Lovibond colour, a wattle bark characteristic that plays a significant role in the wattle extract production for the leather tanning industry (Hillis, 1997).

In Figure 6.9 (A–E), clonal variety responses at Harden Heights and Schwarzwald further show the influence of site conditions on bark quality traits. Bark samples from the Schwarzwald (CLT2) trial had slightly higher Extractives % and Tannin % than samples from Harden Heights (CLT3). Samples from Luneburg also showed 20% higher values for Non-Tannin % than equivalent samples from Harden Heights (CLT3), suggesting a strong site-by-genotype/clone interaction. This can be further explored with in-depth statistical analysis. Differences between clonal varieties at the same site were also noted. SP6-10 was observed to have good Tannin % and one of the lowest Lovibond Red colour at both sites, performing better than the other varieties. Although the Schwarzwald (CLT2) site had Iron (Fe) rich, sandy soil, which are known contributors to darkened bark Lovibond Colour (Avadianund Bridglall *et al.,* 2025), varieties such as SP6-10, SP4-39, SP6 -67, and SP6-96 showed no variation in colour between the samples taken from the Schwarzwald (CLT2) and Harden Heights (CLT3) trials. This suggests that plant genotypes are influenced by the site conditions, but that the genotypic response is dominant (Van Eeuwijk *et al*.,2016).

The PCA biplots demonstrate that while bark quality parameters are intrinsically and consistently intercorrelated across all sites, their relationships with soil properties are neither uniform nor structurally consistent. This pattern provides clear evidence of genotype-by-environment interaction and cautions against attributing bark chemistry variation to edaphic factors alone. At Creydt (Figure 6.10), strong alignment among Extractives %, Tannin %, and colour parameters indicates coordinated biochemical regulation. The opposing orientation of Sand % relative to these traits, and the positive alignment with Clay %, suggests that soil texture contributes meaningfully to bark quality variation at this site. However, this relationship appears site-specific rather than generalizable, as similarly strong soil–bark alignment is not replicated elsewhere.

At Luneburg (Figure 6.11), soil vectors exhibit shorter lengths and weaker directional agreement with bark quality traits, indicating reduced explanatory power. Although Extractives % align positively with Clay % and negatively with Sand %, Mn, and Fe, these relationships are moderate and not structurally dominant within the ordination space. P displays contrasting associations with colour and tannin, further underscoring nutrient-specific rather than system-wide soil effects.

In the clonal trial at Schwarzwald (CLT2) (Figure 6.12), soil–bark relationships are notably weak. Clay % shows no meaningful association with Extractives % or Tannin %, and the limited alignment between soil vectors and bark traits suggests that genetic control outweighs environmental influence under these conditions. The strong clustering of bark quality variables, independent of soil gradients, reinforces this interpretation.

A similar pattern is evident at Harden Heights (CLT3) (Figure 6.13), where bark traits remain tightly grouped while soil parameters define opposing but weakly explanatory gradients. The absence of consistent soil alignment across clonal sites indicates that soil properties are insufficient to account for bark chemistry variation in genetically structured populations.

Collectively, these results demonstrate that soil effects are context dependent and strongest only under specific site conditions (e.g., Creydt). The stability of internal bark quality relationships across all environments suggests intrinsic biochemical coordination, whereas variable soil alignment confirms that environmental

modulation is secondary and site-specific. Therefore, bark quality variation should be interpreted primarily through a genotype by environment framework rather than as a direct function of soil composition alone.

This study has highlighted the potential impact of NIRS on breeding black wattle as a tool for measuring bark quality parameters across multiple varieties at various sites. Measurements can be done for the entire life of the tree before harvesting to determine the effects of other parameters such as climate change, rainfall, etc.

Although the benchtop NIR has shown excellent potential as a tool to analyse bark quality, one of its disadvantages is that a physical sample needs to be taken of the bark. Some of the trees at the Schwarzwald site experienced gummosis after the sampling, which damaged the trees, as seen in Figure 6.14.

Figure 6.14 Gummosis affecting wattle trees at the Schwarzwald site after bark sampling in April 2022 (Photo: J. Moreno Chan).

The development of a completely non-destructive bark analysis method using a low-cost handheld NIR device could potentially address challenges associated with conventional bark sampling. This method allows for the surface of the bark to be scanned, instead of bark samples being taken, causing damage to the tree. One limitation, however, is that handheld instruments typically operate within narrower

spectral ranges compared to benchtop NIRS systems, which cover a broader wavelength spectrum. Despite this constraint, portable NIRS devices are already widely applied in the animal feed industry for the rapid assessment of forage quality indices (Rukundo *et al.,* 2021), demonstrating their practical field applicability.

The adoption of a streamlined handheld NIRS approach within the black wattle industry could enable high throughput screening of large breeding and field trial populations through infield scanning of standing trees. Such capability would enhance decision making processes and support more targeted, data driven breeding and research initiatives. In addition, the technology offers a dependable method for screening and identifying superior black wattle parents and clones, facilitating the selection of genotypes best suited to specific site conditions.

Overall, the implementation of NIRS within breeding programmes, such as those of the ICFR, has the potential to improve the efficiency of selecting superior germplasm, ultimately contributing to enhanced timber and bark yield through the deployment of high-quality genetic material.

6.6 References

Acquaah, G. 2015. Conventional plant breeding principles and techniques. *Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools.* Publisher, City, Country. pp.115-158.

Avadianund Bridglall, P., Laing, M., Naidoo, N., and Burgdorf, R. 2024. A novel laboratory method for the extraction of black wattle (*Acacia mearnsii* de Wild.) bark constituents. *Journal of American Leather Chemists Association*,120(10), pp 26 – 41.

Avadianund Bridglall, P., Laing, M., Morris, A., and Burgdorf, R. 2025. A review of the factors affecting black wattle (*Acacia mearnsii* De Wild.) bark quality in South Africa. *Southern Forests: A Journal of Forest Science.* (Accepted April 2025). In Press.

Avadianund Bridglall, P.A., Laing, M.L., Morris, C. and Burgdorf, R.J. 2025. Ageing, climatic, physical, and site effects on black wattle (*Acacia mearnsii* de Wild.) bark. View online at Wiley Online Library (wileyonlinelibrary.com); DOI:10.1002/bbb.2795; *Biofuels, Bioproducts and Biorefining.* Pp 1- 17.

Avadianund Bridglall, P., Laing, M., and Burgdorf, R. 2025. Near-Infrared Spectroscopic (NIRS) analysis of black wattle (*Acacia mearnsii* de Wild.) bark quality parameters. *Journal of the Science of Food and Agriculture.* Submitted May 2025. Under Review.

Bairu, M.W., Amelework, A.B. and Coetzer, W.G. 2021. Genetic diversity and population structure of six South African Acacia mearnsii breeding populations based on SSR markers. *Journal of Plant Research,* 134(6), 1243-1252.

Beck, S.L., de Guisti, J., Louw, A.K. and Resende, M.D.V. 2007. Assessment of three-year DBH and height data and genetiRc gain prediction for five *Acacia mearnsii* (black wattle) subpopulations in South Africa using REML/BLUP. *Southern Hemisphere Forestry Journal*, *69*(2), 111-116.

Booth, T.H. 1997. Climatic Factors. In Brown, A.G., and Ko, H.C. (Eds): *Black Wattle and Its Utilisation*. Rural Industries Research and Development Corporation, Canberra. Pp 13 -18.

Brady, N.C., and Weil, R.R. 2008. *The Nature and Properties of Soils.* Prentice Hall, Upper Saddle River, NJ. Vol. 13, pp. 662-710.

Burgdorf, R., and Germishuizen, I. 2021. Climatic effects on factory Mimosa extract quality. Technical Report, *Institute for Commercial Forestry Research* (Unpublished).

Cen, H. and He, Y. 2007. Theory and application of near infrared reflectance spectroscopy in determination of food quality. *Trends in Food Science & Technology*, *18*(2), 72-83.

Cozzolino, D. 2009. Near infrared spectroscopy in natural products analysis. *Planta Medica*, *75*(07), 746-756.

Donkin, M.J., Pearce, J., and Chetty, P.M. 1993. *Methods for Routine Plant Analysis in the ICFR Laboratories.* Bulletin Series 6/93, ICFR, Pietermaritzburg.

Dunlop, R., 1998. *Final report on two Australian Acacia mearnsii (black wattle) provenance trials established in KwaZulu-Natal and south-eastern Mpumalanga.* ICFR Bulletin Number 7/98. Institute for Commercial Forestry Research. Pp 41-45.

Dunlop, R.W. 2002. *Tree Improvement.* In: Dunlop, R.W. and MacLennan L.A. (Eds). *Black Wattle: The South African Research Experience*. Institute for Commercial Forestry Research. Pietermaritzburg.

Dunlop, R.W., Resende, M.D.V. and Beck, S.L. 2005. Early assessment of first year height data from five *Acacia mearnsii* (black wattle) sub-populations in South Africa using REML/BLUP. *Silvae Genetica*, *54*(4-5), 166-173.

Gepts, P. and Hancock, J. 2006. The future of plant breeding. *Crop Science*, *46*(4), 1630-1634.

Gibson, K.S. 1927. *The Lovibond Color System:* A spectrophotometric analysis of the Lovibond glasses (No. 547). US Government Printing Office, Washington. Pp 4 - 7.

Gordon-Gray, C.G. 1953. A rapid method of determining the relative tannin content of young, living black wattle trees in genetic and breeding experiments. *Report-Wattle Research Institute*. Pp 42-48.

Griffin A.R., Midgley S.J., Bush D., Cunningham P.J., Rinaudo, T.T., Kelly R.M., Harbard J.L., Moreno Chan, J. 2023. Global Uses of Australian Acacia Species: Recent Trends and Future Prospects. In: Richardson, D.M., Le Roux J.J., Marchante, (Eds). Australian Acacia Species Around the World. CAB International. United Kingdom.

Hagerman, A.E., 2002. *The Tannin Handbook*. The Department of Chemistry and Biochemistry, Miami University and Oxford. Pp 1-6.

Hayward, M.D., Bosemark, N.O. and Romagosa, T. 2012. *Plant Breeding: Principles and Prospects*. Springer Science & Business Media. Pp. 3-14.

Hemingway, R.W. 2018. Bark: Its chemistry and prospects for chemical utilization. In: F.A. Vajhidi and C.E. Wyman, eds. In *Organic Chemicals from Biomass*. Bocs Raton: *CRC Press.* Pp. 189-248.

Hillis, W.E. 1997. Tannin chemistry. In Brown, A.G., and Ko, H.C. (Eds): *Black Wattle and its Utilisation*. Rural Industries Research and Development Corporation, Canberra, Australia. Pp*106*.

https://www.statstodo.com/LinCCC.php. Accessed April 2025.

Jain, R., Janakiram, T. and Kumawat, G.L. *Drying Techniques in Ornamental - Plants.* Division of Horticultural Science, Indian Agricultural Research Institute, New Delhi, India. Pp 501-512, 2016.

Kays, S.E., Barton, F.E., and Windham, W.R. 2000. Predicting protein content by near infrared reflectance spectroscopy in diverse cereal food products. *Journal of Near Infrared Spectroscopy*, *8*(1), 35-43.

Lawrence, I. and Lin, K. 1989. A concordance correlation coefficient to evaluate reproducibility. *Biometrics*, (45)1, 255-268.

Leafe, M.K. (Ed.). 1999. *Leather Technologist's Pocketbook.* Society of Leather Technologists and Chemists, United Kingdom. Pp 227 -272.

Miller, C.E. and Igne, B. 2021. Preprocessing methods in NIR spectroscopy. In Ciurczak, E.W., Igne, B., Workman, Jr., J., and Burns, D.A. (Eds.). (2021). *Handbook of Near-Infrared Analysis* (4th ed.). CRC Press. Pp 285 - 289.

Ciurczak, E.W., Igne, B., Workman, Jr., J., & Burns, D.A. (Eds.). (2021). Handbook of Near-Infrared Analysis (4th ed.). CRC Press.

Moose, S.P. and Mumm, R.H. 2008. Molecular plant breeding as the foundation for 21st century crop improvement. *Plant Physiology*, *147*(3), 969-977.

Moreno Chan, J., Day, P., Feely, J., Thompson, R., Little, K.M. and Norris, C.H. 2015. Acacia mearnsii industry overview: current status, key research and development issues. *Southern Forests: A Journal of Forest Science*, 77(1), 19-30.

Moreno Chan J, and Isik F (2019) Genetic variation in resistance to *Uromycladium acaciae* fungus, growth, gummosis and stem form in *Acacia mearnsii* Populations. *Tree Genetics and Genomes*, 15:1-13, DOI 10.1007/s11295-019-1341-x.

Moreno Chan J. 2019. Frost tolerance of six seed orchards of *Acacia mearnsii* (black wattle) and the effect of developmental stage and tree size on frost hardiness. *Australian Forestry*, 82:1, 35-47, DOI 10.1080/00049158.2019.1583112.

Olivoto, T., Nardino, M., Carvalho, I.R., Follmann, D.N., Szareski, V.J., Ferrari, M., de Pelegrin, A.J. and de Souza, V.Q., 2017. Plant secondary metabolites and its dynamical systems of induction in response to environmental factors: A review. *African Journal of Agricultural Research*, *12*(2), 71-84.

Ozaki, Y. 2022. NIR spectroscopy–What a wonderful world! *NIR News*, *33*(1-2), 10-17.

Poynton, R.J. 2003. Exotic trees: their significance in the South African context: guest editorial. *Southern African Forestry Journal*, *2003*(197),1-4.

Rukundo, I.R., Danao, M.G.C., Mitchell, R.B., Masterson, S.D. and Weller, C.L., 2021. Comparing the use of handheld and benchtop NIR spectrometers in predicting nutritional value of forage. *Applied Engineering in Agriculture*, *37*(1), 171-181.

Schimleck, L.R. and Yazaki, Y., 2003. Analysis of black wattle (*Acacia mearnsii* De Wild) bark by near infrared spectroscopy. *Holzforschung*, (57)5, 527-532.

Schonau, A.P.G. 1970. The effect of fertilizer, lime, and trace element applications on growth and disease incidence in black wattle (*Acacia mearnsii de Wild*). Report. Wattle Research Institute, University of Natal, 1971.Pp 38-48.

Sewpersad, Y., 2004. *Estimation of Genetic Variation and Marker Identification in Black Wattle (Acacia mearnsii De Wild) with RAPD Fingerprinting*. Ph.D. thesis, University of KwaZulu-Natal. Pp 15 -25.

Slabbert, N. 1992. Complexation of condensed tannins with metal ions. In *Plant Polyphenols*. Basic Life Sciences, Springer, Boston, MA, USA. Vol 57, pp 421 – 436.

Van Eeuwijk, F.A., Bustos-Korts, D.V., and Malosetti, M. 2016. What should students in plant breeding know about the statistical aspects of genotype × environment interactions? *Crop Science*, *56*(5), 2119-2140.

Wang, Y., Xiang, J., Tang, Y., Chen, W. and Xu, Y., 2022. A review of the application of near-infrared spectroscopy (NIRS) in forestry. *Applied Spectroscopy Reviews*, *57*(4), 300-317.

Williams, P., and Norris, K. Near-infrared technology in the agricultural and food industries. *American Association of Cereal Chemists, Inc.* Pp 220 – 330.

Thesis Overview

Introduction

Black wattle (*Acacia mearnsii* De Wild.) is a commercially important forestry species in South Africa, valued for both its timber and high-quality condensed tannins derived from its bark (Nair, 2021). The industry contributes significantly to the national economy, generating approximately USD 60 million annually (Chan *et al.*, 2015). However, concerns regarding declining bark extract quality particularly darkening of extracts and variability in tannin content have raised questions about long term industry sustainability. These changes which can be associated with climatic variability, altered silvicultural practices, shifts in plantation management, and reduced research investment.

This thesis was initiated in collaboration with industry stakeholders to address critical knowledge and analytical gaps. The primary objectives were to:

- Develop rapid, high-throughput analytical tools for bark quality determination.
- Identify environmental, genetic, and processing factors contributing to bark extract darkening.
- Provide scientifically grounded mitigation strategies to strengthen industry sustainability and competitiveness.

Collectively, the research integrates analytical chemistry, spectroscopy, silviculture, breeding, and environmental assessment to provide a comprehensive framework for improving bark extract quality. The key findings are noted for each chapter.

Chapter 1: A review of the production and factors affecting black wattle (Acacia mearnsii De Wild.) bark and bark extract quality in South Africa

Chapter 1 provides a critical review of the historical development, current status, and future trajectory of the black wattle industry in South Africa. It synthesises literature spanning silviculture, bark chemistry, harvesting practices, extract processing, and global market trends.

The key findings of the literature review are listed below:

- A documented decline in black wattle plantation area (ICFR, 2017) and associated research investment.
- A growing international shift toward diversified tannin applications beyond leather, including pharmaceuticals, adhesives, and feed additives (Pizzi, 2008).
- Continued reliance in South Africa on traditional leather tanning markets.
- Limited contemporary research addressing bark colour stability and climate-related impacts.

The review highlights a strategic gap between international innovation and local industry focus. It concludes that revitalisation of research, diversification of applications, and proactive management of plantation decline are essential to ensuring the industry's long-term resilience.

Chapter 2: A novel laboratory method for the extraction of black wattle (*Acacia mearnsii* de Wild.) bark constituents

The traditional Soxhlet and SLTC analytical methods used for tannin determination are time consuming, labour-intensive, and insufficient for evaluating extract colour stability (Havemann, 1992). Furthermore, these methods do not adequately simulate industrial extraction conditions, where wattle bark is extracted in large autoclaves (Das *et al.*, 2020).

The key findings of Chapter 2 are listed below:

- Development of the Double Autoclave Water (DAW) extraction method to improve analytical throughput.
- Enhanced preservation of extract colour compared to traditional reflux-based methods.
- Improved alignment between laboratory extraction and industrial autoclave processes.
- Demonstration that freeze-drying reduces enzymatic degradation and preserves tannin integrity (Oetjen and Haseley, 2004).
- Identification of ethylenediaminetetraacetic acid (EDTA) as a colour improving chelating agent. However, EDTA has been banned by Australia and certain western Europe countries as a food additive (Sinex, 2004). Therefore, research

into the use of less toxic reducing or chelating agents should be explored, as a tool to enhance the quantity and quality of tannins extracted from wattle bark.

The DAW method represents a practical and scalable analytical advancement, providing a more accurate reflection of factory extraction performance while significantly increasing laboratory efficiency.

Chapter 3: Near-Infrared Spectroscopic (NIRS) analysis of black wattle (*Acacia mearnsii* de Wild.) bark quality parameters

Near Infrared Spectroscopy (NIRS) has gained widespread popularity as a versatile tool used for many analytical applications. Davies (1998) stated that, "The giant is running strong", when referring to NIRS due to the exponential growth in NIRS applications in many fields, including agricultural, petrochemical, pharmaceutical, clinical, and environmental analyses (Blanco and Villarroya, 2002). Therefore, developing NIRS for the determination of wattle bark quality parameters is long overdue.

Chapter 3 addresses the analytical bottleneck associated with destructive and chemically intensive reference methods such as the SLTC method (the industry's "Golden Standard") for tannin analysis (Yazaki *et al.*,1993). NIRS was evaluated as a rapid, non-destructive alternative capable of predicting multiple bark quality parameters.

The key findings of Chapter 3 are listed below:

- NIRS analysis of liquid extracts, using transmission cell and transflectance stamp approaches, calibrated against SLTS data, is feasible.
- The development of robust calibration models with strong predictive accuracy ($R^2 > 0.80$).
- The use of freeze-dried samples to eliminate water interference in spectral models. Powder samples could also be developed in a similar manner.
- Prediction of more than 20 bark quality parameters directly from solid bark.
- Strong agreement between NIRS predictions and reference chemistry in independent validation sets.

This work has provided a solid foundation to justify the use of NIRS by the industry; future research needs to explore the potential for direct analysis of wattle bark chips at factories without any sample pre-processing. The use of new, low-cost portable devices, or in-line NIRS monitors of conveyor belts carrying bark chips to monitor bark quality bark consignments arriving at depots and factories could be developed to improve product quality control. In addition, the inline use of NIRS could be used to optimize bark extraction operations in the wattle bark factories of the various liquid extracts (Donkin and Pearce, 1995).

Chapter 4: Ageing, climatic, physical, and site effects on black wattle (*Acacia mearnsii* de Wild.) bark

Climate Change is a global threat to the agricultural industry due to its impact on the physiological and metabolic activities of plants, resulting in changes in plant growth, productivity, quality of products, and pest infestation (Mahi *et al.*, 2021).

This chapter investigated environmental drivers of bark quality degradation, particularly in the context of climate change. The study integrated climatic variables, soil characteristics, ageing effects, and physical treatments to identify factors influencing extract colour and tannin content.

The key findings of Chapter 4 are listed below:

- Solar radiation identified as the primary driver of extract colour degradation, it is known to affect tree physiology (Phillips and Riha, 1993).
- Significant influence of soil texture and nutrient composition on bark chemistry.
- Evidence that environmental stress exacerbates bark quality variability.
- Recommendation for long-term site monitoring and adaptive silvicultural management.

Long-term site monitoring would enable informed decision-making regarding fertiliser application, identification of environmental factors influencing bark quality at each site, and targeted control of pests and diseases (Mead, 2005). The findings highlight the importance of understanding genotype x environment interactions and implementing robust monitoring systems to mitigate climate driven declines in bark quality. Continuous plantation monitoring throughout the tree lifecycle would further support proactive and adaptive silvicultural management.

Chapter 5: Investigations on the effects of green wattle (*Acacia decurrens* var. *dealbata* (Link) F. Muell.) bark on predominantly black wattle (*Acacia mearnsii* De Wild) bark extract quality, and its detection in mixed bark consignments

The differences between black and green wattle makes one question as to why green wattle is not considered to be the superior species. The many favourable qualities of green wattle have been noted in the literature and observed in this study. However, if the main application of wattle bark for the tannin industry is to produce chemicals to tan leather, then green wattle is unsuitable due to the lower tannin content and darker red colour of its bark extracts (Coetzee, 1986).

Black wattle bark extract is widely recognised for its high tannin content and pale colour, which contributes to its effectiveness in leather tanning (Havemann, 1992). In contrast, green wattle bark extract has lower tannin levels and a darker red colour, which reduces its commercial viability (Craib, 1941). Despite this, green wattle is often favoured in reforestation and erosion control due to its rapid growth, resistance to pest and diseases, and timber quality. (Midgley & Turnbull, 2003 and Marcar *et al*., 1995).

The key findings of Chapter 5 are listed below:

- It was demonstrated that up to 40 % green wattle inclusion did not significantly reduce tannin content or colour quality.
- The development of NIRS models capable of discriminating between the two species.
- The quantitative prediction of green wattle proportion in mixed consignments.

The findings of this study underscore the importance of analytical tools such as NIRS for quality assurance in the bark extract industry. Given the observed decline in black wattle plantation areas in South Africa (Morris, 2022), alternative uses for green wattle bark, such as in feed additives, adhesives, or biochemicals should be explored through further research.

This work contributes to the sustainable development of the wattle industry by supporting species differentiation, quality monitoring, and broader industrial utilisation strategies. It also builds foundational knowledge for managing raw material integrity in high-value export commodities derived from bark extract.

Chapter 6: Application of Near-Infrared Spectroscopy (NIRS) to estimate the bark quality of black wattle seed sources and clones

This study explored the application of Near-Infrared Spectroscopy (NIRS) as a semi-destructive tool to predict bark quality parameters in black wattle (*Acacia mearnsii*) breeding trials across multiple sites in South Africa. Traditional SLTC methods used for bark quality analysis are destructive, time-consuming, and sample-intensive, making them impractical for large-scale screening (Gordon – Gray, 1953). The NIRS application and the models developed on wattle bark samples from seed source block trials and clonal trials produced excellent results, whereby one set of models was created for all varieties and was used to predict accurately the performance of almost 300 samples, saving time and costs associated with chemical analyses.

The key findings of Chapter 6 are listed below:

- Unified calibration models accurately predicted bark quality across seed source and clonal trials ($R^2 > 0.98$).
- Bark quality differed between sites.
- Genotype-by-environment interactions influenced bark chemistry.
- In clonal trials, genetic effects were stronger than soil effects.
- Certain clones (e.g., SP6-10) consistently performed well across sites.
- NIRS shows strong potential as a rapid screening tool for black wattle breeding programmes.
- Non-destructive handheld NIRS for future field applications to be further developed.

Overall, NIRS was shown to be a powerful tool for rapid, accurate, and cost-effective bark quality screening in black wattle breeding. It facilitates early selection, optimised site allocation, and genetic improvement, supporting sustainable forestry practices in South Africa (Wang *et al*., 2022; Walker and Panozzo, 2023).

Conclusion

This thesis delivers an integrated scientific framework for improving black wattle bark extract quality in South Africa, by combining methodological innovation, environmental assessment, species evaluation, and breeding applications, the work provides both immediate industrial solutions and long-term strategic guidance.

The favourable outcome of this research is as follow:

- Introduction of the DAW method for rapid, colour-preserving extraction.
- Validation of NIRS as a transformative, non-destructive analytical platform.
- Identification of climatic and site-related drivers of bark quality decline.
- Establishment of quantitative tools for managing mixed species consignments.
- Demonstration of NIRS as a powerful breeding and selection tool.

Collectively, the research shifts bark quality analysis from reactive laboratory testing to proactive, predictive monitoring. It establishes Near Infrared Spectroscopy as a central technology for quality assurance, climate adaptation, and genetic improvement, thereby strengthening the long-term sustainability and global competitiveness of the South African black wattle industry.

This work has also brought to focus the need for continual monitoring programs. Once the major factors have been identified, mitigation strategies such as breeding programs or adapted silvicultural practices, can be developed to factor in wattle bark quality, in addition to optimal and sustainable timber production.

References

Blanco, M. and Villarroya, I.N.I.R. 2002. NIR spectroscopy: a rapid-response analytical tool. *TrAC Trends in Analytical Chemistry*, *21*(4), 240-250.

Chan, J.M., Day, P., Feely, J., Thompson, R., Little, K.M., and Norris, C.H. 2015. *Acacia mearnsii* industry overview: current status, key research, and development issues. *Southern Forests: A Journal of Forest Science*, *77*(1), 19-30.

Coetzee, J., 1986. A comparison of bark yield and pole utilisation between black wattle (*Acacia mearnsii*) and green wattle (*A. decurrens*). *Annual Report, Institute for Commercial Forestry Research, University of Natal*, pp.133-139.

Craib, I.J. 1941. South African wattle bark and wattle extract with special reference to the American market. *Journal of the South African Forestry Association*, *6*(1), pp.71-88.

Das, A.K., Islam, M.N., Faruk, M.O., Ashaduzzaman, M. and Dungani, R. 2020. Review on tannins: Extraction processes, applications, and possibilities. *South African Journal of Botany*, *135*, 58-70.

Davies, T., 1998. The history of near infrared spectroscopic analysis: Past, present and future" From sleeping technique to the morning star of spectroscopy". *Analysis*, *26*(4),17-19.

Donkin, M.J. and Pearce, J. 1995. Tannin analysis by near-infrared spectroscopy. *Journal of the Society of Leather Technologists and Chemists*, *79*(1), 8-11.

Havemann, D.A.1992. *The Manufacture and Uses of Wattle Bark Extract*. Ph.D. thesis, University of Stellenbosch. Pp 17-48.

Gordon-Gray, C.G. 1953. A rapid method of determining the relative tannin content of young, living black wattle trees in genetic and breeding experiments. *Report-Wattle Research Institute*. Pp 42-48.

ICFR Bulletin. 2017. Honouring the past, celebrating the present, and innovating the future. Pp 1-4.

Marcar, N., Crawford, D., Leppert, P., Jovanovic, T., Floyd, R. and Farrow, R. 1995. *Trees for saltland: A guide to selecting native species for Australia*. CSIRO Publishing.

Malhi, G.S., Kaur, M. and Kaushik, P. 2021. Impact of climate change on agriculture and its mitigation strategies: A review. *Sustainability*, *13*(3),1318.

Mead, D.J. 2005. Opportunities for improving plantation productivity. How much? How quickly? How realistic? *Biomass and Bioenergy*, *28*(2), 249-266.

Midgley, S.J. and Turnbull, J.W. 2003. Domestication and use of Australian acacias: case studies of five important species. *Australian Systematic Botany*, *16*(1), pp.89-102.

Morris, A.R. 2022. Changing use of species and hybrids in South African forest plantations. *Southern Forests: A Journal of Forest Science*, *84*(3), 193-205.

Nair, K.P. 2021. Wattle. In *Tree Crops*. Springer, Cham. 363 – 375.

Oetjen, G.W. and Haseley, P. 2004. *Freeze-drying*. John Wiley & Sons. Pp 2-10.

Phillips, J.G. and Riha, S.J., 1993. Canopy development and solar conversion efficiency in *Acacia auriculiformis* under drought stress. *Tree Physiology*, *12*(2), pp.137-149.

Pizzi, A. 2008. Tannins: major sources, properties, and applications. In: *Monomers, Polymers, and Composites from Renewable Resources*. M.N. Belgacem and A. Gandini (Eds). Elsevier Science. pp. 179-199.

Sinex, S.A. 2004. EDTA-A Molecule with a Complex Story. *The University of Bristol, School of Chemistry website (accessed Jan 8, 2013) (Aug 1, 2007)*.

Walker, C.K. and Panozzo, J.F., 2023. Near-infrared spectroscopy enables quality selection in wheat breeding. *Cereal Chemistry*, *100*(6), 1347-1356.

Wang, Y., Xiang, J., Tang, Y., Chen, W. and Xu, Y., 2022. A review of the application of near-infrared spectroscopy (NIRS) in forestry. *Applied Spectroscopy Reviews*, *57*(4), 300-317.

Yazaki, Y., Gu, R., Lin, Y., Chen, W. and Nguyen, N. 1993. Analyses of black wattle (*Acacia mearnsii*) tannins: relationships among the hide-powder, the Stiasny, and the ultra-violet (UV) methods. *Holzforschung*, *47*(1), 57-61.

Supplementary Data (Chapter 3 Calibration NITS and NIRS calibration data)

The supplementary data file includes the calibration datasets developed in the OPUS Quant software (version 8.5, Bruker, Germany) based on partial Least Squares Regression (PLSR). The calibration data is provided to support the completeness and transparency of the method development process in Chapter 3.

Table 1 NITS calibration model performance for 10 bark quality parameters on bark extract solutions using a transmission cell for measurement.

Parameter	Unit	Range (Min)	Range (Max)	Spectral Range	R^2	RMSEP	RPD	IQR	RPIQ
Extractives	%	47.1	53.6	9947.7 - 7394.2	80.39	0.70	1.99	6.5	9.3
Tannin	%	30.5	41.3	8246.6 - 7394.2	92.41	0.69	3.28	10.8	15.7
Non-Tannin	%	10.2	15.6	9099.1 - 7394.2	91.63	0.51	3.43	5.4	10.6
Insoluble	%	1.1	3.4	10796.2 - 8246.6	86.91	0.22	2.01	2.3	10.5
Tannin/Non-Tannin Ratio		2.1	3.9	9091- 7394.2	82.33	0.19	2.87	1.8	9.5
Polyphenolics	%	25.9	38.0	11644.8 - 10792.2 & 9947.7 - 7394.2	75.96	2.73	2.71	12.1	4.4
Lovibond Red	LRU	0.9	1.8	12493.4 - 11641	61.47	0.17	1.53	0.9	5.3
Lovibond Yellow	LYU	1.6	3.2	9091- 7394.2	69.84	0.20	1.82	1.6	8.0
Turbidity	NTU	201	371	8246 - 7394.2	60.13	271	161	170	0.6
pH	pH units	4.8	6.0	10796.2 - 7394.2	74.11	0.09	1.82	1.2	13.3

Table 2 NITS calibration performance on 10 bark quality parameters for bark extract solutions using a transflectance stamp for measurement.

Parameter	Unit	Range (Min)	Range (Max)	Spectral Range	R^2	RMSEP	RPD	IQR	RPIQ
Extractives	%	47.04	53.55	7151.1 - 4242.8	63.34	1.04	1.65	6.5	6.3
Tannin	%	30.51	41.31	6102 - 4597.7	38.89	1.77	1.28	10.8	6.1
Non-Tannin	%	10.17	15.60	6102 - 4242.8	68.03	0.81	1.77	5.4	6.7
Insoluble	%	1.06	3.38	7151.1 - 4242.8	25.10	0.50	1.16	2.3	4.6
Tannin/Non-Tannin Ratio		2.09	3.85	6102 - 5446.3 & 4605.4 - 4242.8	51.94	0.30	1.44	1.8	5.9
Polyphenolics	%	25.9	37.95	6102 - 4597.7	64.98	3.03	1.69	12.1	4.0
Lovibond Red	LRU	0.91	1.83	4428 - 4242.8	43.03	0.23	1.31	0.9	4.0
Lovibond Yellow	LYU	1.62	3.18	7151.1 - 6618.8 & 5454 - 4242.8	39.28	0.34	1.28	1.6	4.6
Turbidity	NTU	201	371	7151.1 - 4242.8	91.25	13.00	3.38	170	13.1
pH	pH units	4.78	5.97	6102 - 5446.3 & 4428 - 4242.8	38.05	0.18	1.27	1.2	6.6

Table 3 NIRS calibration model performance of 26 bark quality parameters for freshly collected and processed milled freeze-dried bark.

Parameter	Unit	Range (Min)	Range (Max)	Spectral Range	R^2	RMSEP	RPD	IQR	RPIQ
Freeze-dried Moisture	%	37.1	48.1	7234.7 -4242.8	97.09	0.57	5.86	11.0	19.3
Diameter at Breast Height (DBH)	cm	10.6	16.9	6102 -4242.8	86.00	0.73	2.67	6.3	8.6
Extractives	%	47.04	53.55	7234.7 -4242.8	93.77	0.49	4.01	6.5	13.3
Tannin	%	30.51	41.31	7454- 4242.8	93.57	0.74	3.94	10.8	14.6
Non-Tannin	%	10.17	15.60	6102 -4242.8	93.57	0.42	3.81	5.4	12.9
Insoluble	%	1.06	3.38	6102.0 - 4242.8	81.66	0.31	2.34	2.3	7.5
Tannin/Non-Tannin Ratio		2.09	3.85	6672.8 - 6094.3	97.84	0.09	6.80	1.8	19.6
Polyphenolics	%	25.9	37.95	5454.0 - 4242.8	89.70	1.68	3.12	12.1	7.2
Lovibond Red	LRU	0.91	1.83	7234.7 - 4242.8	99.27	0.03	11.7	0.9	30.7
Lovibond Yellow	LYU	1.62	3.18	7234.7 - 4242.8	98.06	0.07	7.18	1.6	22.3
Turbidity	NTU	201	371	6102.0 - 4597.7	81.73	19.00	2.34	170.0	8.9
pH	pH units	4.78	5.97	5454.0 - 4242.8	86.36	0.10	2.71	1.2	11.9
Al	mg kg^{-1}	74.23	736	7234.7 - 4242.8	93.60	42.50	3.95	662	15.6
B	mg kg^{-1}	8.07	11.19	5454.0 - 4597.7	77.89	0.42	2.13	3.1	7.4
Ca	mg kg^{-1}	6970	13044	7234.7 - 4242.8	97.07	375	5.84	6074	16.2
Cu	mg kg^{-1}	0.87	2.96	7234.7 - 4242.8	97.12	0.11	5.89	2.1	19.0
Fe	mg kg^{-1}	45.70	505	7234.7 - 4242.8	94.66	26.50	4.33	459	17.3
K	mg kg^{-1}	1325	3450	7234.7 - 4597.7	77.41	301	2.1	2125	7.1
Mg	mg kg^{-1}	497	1280	7234.7 - 4242.8	82.41	125	2.38	783	6.3
Mn	mg kg^{-1}	25.04	60.15	7234.7 - 4242.8	95.53	2.04	4.73	35.1	17.2
Na	mg kg^{-1}	60.91	259	7234.7 - 4242.8	82.94	24.40	2.42	198.	8.1
P	mg kg^{-1}	0	228	7234.7 - 6094.3	89.92	26.20	3.15	228	8.7
Zn	mg kg^{-1}	0	8.80	7234.7 - 4242.8	80.01	1.16	2.24	8.8	7.6
C	%	47.06	50.86	7234.7 - 4242.8	88.77	0.35	2.98	3.8	10.9
N	%	0.78	1.15	6102.0 - 4242.8	91.20	0.03	3.37	0.4	12.3
S	%	0.02	0.06	7234.7 - 6094.3 &5454.0 -4242.8	93.98	0.003	4.08	0.0	13.3

Table 4 NIRS calibration model performance for treated (aged, wet/dry, high/low temperature, and light/dark exposure) freeze-dried, and milled bark. The model was created to determine the eight priority bark quality parameters.

Parameter	Unit	Range (Min)	Range (Max)	Spectral Range	R^2	RMSEP	RPD	IQR	RPIQ
Extractives	%	43.7	55.8	5338.3 - 4381.7 & 3911.1 - 3749.1	78.71	0.51	2.63	12.1	23.7
Tannin	%	26.6	41.3	5338.3 - 3749.1	88.16	0.77	3.48	14.7	19.1
Non-Tannin	%	10.6	18.9	5338.3 - 4219.7	70.43	0.83	2.45	4.2	5.0
Insoluble	%	0.9	3.5	5338.3 - 3904.3	22.09	0.50	2.01	2.6	5.2
Tannin/Non-Tannin Ratio		1.2	3.3	5338.3 - 4543.7	78.93	0.17	1.98	2.1	12.4
Manual Lovibond Red	LRU	0.8	3.0	5338.3 - 3749.1	94.18	0.08	4.23	2.2	27.5
Manual Lovibond Yellow	LYU	1.5	5.8	5338.3 - 4543.7	90.40	0.14	2.87	4.3	30.7
Turbidity		119	371	5184.0 - 3749.1	86.62	9.11	2.51	252	27.7

Table 5 NIRS calibration model performance for chipped and chopped factory milled freeze–dried bark. The model was created for 21 bark quality parameters.

Parameter	Unit	Range (Min)	Range (Max)	Spectral Range	R^2	RMSEP	RPD	IQR	RPIQ
Extractives	%	44.3	59.1	7506 - 6094.3 & 5454 -4242.8	98.63	0.29	8.54	14.8	51.0
Tannin	%	32.6	37.4	7506 - 4242.8	99.96	0.02	4.89	4.8	240
Non-Tannin	%	10.7	16.6	6102 - 4242.8	98.69	0.32	8.73	5.9	18.4
Insoluble	%	0.6	1.7	6102 - 4242.8	98.41	0.04	7.94	1.1	27.5
Tannin/Non-Tannin Ratio		1.5	3.5	5454 - 4242.8	98.19	0.06	7.43	2.0	33.3
Manual Lovibond Red		0.8	2.2	7506 - 6094.3 & 5454 - 4242.8	99.83	0.01	2.41	1.4	140
Manual Lovibond Yellow		1.3	4.5	7506 - 4242.8	99.76	0.04	20.3	3.2	80.0
Al	mg kg^{-1}	57.4	1506	7506 - 4242.8	95.76	73.40	4.86	1449	19.7
B	mg kg^{-1}	6.9	12.3	7506 - 4242.8	98.78	0.19	9.07	5.4	28.4
Ca	mg kg^{-1}	3637	11749	7506 - 4242.8	98.20	338	7.45	8112	24.0
Cu	mg kg^{-1}	0.9	5.0	9303.7 - 4242.8	98.82	0.10	9.21	4.1	41.0
Fe	mg kg^{-1}	47.01	1115.6	7506 - 4242.8	92.88	59.70	3.75	1069	17.9
K	mg kg^{-1}	2274	5422	6102 - 4242.8	97.34	140	6.13	3148	22.5
Mg	mg kg^{-1}	673	1576	9403.7 - 4597.7	95.32	43.10	4.62	903	21.0
Mn	mg kg^{-1}	17.1	113.1	7506 - 4242.8	95.18	3.02	4.56	96	31.8
Na	mg kg^{-1}	39.2	534	7506 - 4242.8	96.27	16.30	5.17	495	30.4
P	mg kg^{-1}	0	264	7506 - 4242.8	96.21	15.60	5.14	264	16.9
Zn	mg kg^{-1}	0	4.9	9403.7 - 4597.7	88.10	0.45	2.90	4.9	10.9
C	%	47.3	50.8	6102 - 4242.8	97.23	0.17	6.01	3.5	20.6
N	%	0.8	1.2	7506 - 6094.3 & 5454 - 4242.8	97.71	0.02	6.60	0.4	20.0
S	%	0	0.1	7506 - 4242.8	86.99	0.006	2.77	0.1	16.7