Appendix 3. PhD thesis by Amit Khanal

Relationship between fruit maturity and internal disorders of vapour heat treated ‘B74’ mango fruit

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M.Sc. Ag. (Horticulture)

A thesis submitted for the degree of Doctor of Philosophy at

The University of Queensland in 2023

School of Agriculture and Food Sciences

Gatton Campus

Abstract

'B74'(Calypso™) is the second most economically important mango cultivar in Australia. It is generally well received in domestic and overseas markets. However, internal disorders (IDs) that express postharvest represent a challenge for the industry. Understanding of what makes 'B74' mangoes susceptible to expression of IDs is wanting. This thesis investigates factors associated with IDs in 'B74' mango. It sought to recommend strategies to mitigate IDs with regard to export supply chains in which stressful phytosanitary treatments are required.

Initially, a survey was conducted of commercial operations concerned with producing 'B74' fruit for export. It sought commercial insight as to factors potentially predisposing the fruit to express IDs. It was comprised of grower and handler surveys and coupled with supply chain monitoring. The prevalent IDs for 'B74' were Flesh Cavity with White Patches (FCWP) and Flesh Browning (FB). FCWP was clearly induced by phytosanitary vapour heat (VHT) treatment. On the other hand, FB was an issue not contingent upon VHT. Fruit maturity was a putatively important determinant of both IDs. Postharvest handling, in terms of postharvest time and temperature conditions, was also considered a contributing factor.

Hence, the study focused on fruit maturity in relation to the IDs. Three separate experiments were conducted on incidence and severity of IDs. In the first experiment, fruit were sorted into three maturity classes based on dry matter content (represented as a percentage of dry weight to fresh weight; DMC): <15%, 15 - 17%, and >17%. Half in each maturity class were vapour heat treated (VHT) and the remainder were untreated controls. Only FCWP was in this experiment, and it occurred only in VHT fruit. FCWP incidence and severity were greatest *($P<0.05$)* at 12.4% and 0.2, respectively in <15% DMC fruit than in fruit from the higher maturity classes.

For the second experiment, fruit were harvested at early and late maturity stages in 2-weeks interval with average DMC of 14.5% and 17.4%, respectively. For both harvests, fruit were subject to no-VHT, VHT, and VHT following 12 h pre-conditioning at 37 ± 1 °C. Consistent with the previous experiment, FCWP was observed only in VHT fruit. Early harvest fruit exhibited significantly *($P<0.05$)* higher FCWP incidence (26.9%) and severity (0.3) than did late harvest fruit (8.3% incidence and 0.1 severity). Conditioning significantly *($P<0.05$)* reduced FCWP from 45.0% to 7.8%. Conditioning was relatively more effective for early harvested fruit, with seven- and four-fold reductions for early and late harvested, respectively. FB incidence was observed only in late harvested fruit (2. 8%).

In experiment three, the relative efficacy of floatation in 2% salt solution towards maturity sorting was evaluated for IDs incidence. Both 'floaters' and 'sinkers' were subject to VHT and assigned to two sea freight simulations: controlled atmosphere (CA) of 3% O_2 and 5% CO_2 at 11 °C, and ambient atmosphere at 11 °C, both for 21 days. However, DMC did not differ *(P>0.05)* between groups the two groups that evidently differed in density. As flotation was evidently not useful for maturity discrimination with 'B74', FCWP and FB incidences did not differ. However, Storage conditions had significant *(P<0.05)* effect in that FB was reduced from 71.1% under ambient storage to 33.3% under CA.

The efficacy of employing commercial in-line near infra-red (NIR) sorting technology for DMC sorting was subsequently tested. In-line NIR effectively predicted 'B74' mango fruit maturity (DMC) and soluble solids (TSS) (r^2 = 0.8, SEP = 0.7). FCWP binary prediction accuracy was 73%, with sensitivity of 78%. In stark contrast, FB prediction sensitivity was 0%. Nevertheless, relations between IDs and fruit maturity at harvest were clear. Less mature fruit were more susceptible to FCWP. More mature fruit were more susceptible to FB. Thus, sorting fruit using in-line NIR technology could help to ensure that only relatively tolerant fruit are directed, including via VHT, into export market channels.

This study highlights factors, especially DMC, linked to expression of IDs in 'B74' mango fruit. It formulates a practical approach to minimise them in export supply chains, including to protocol markets. In summary, less mature fruit were relatively more susceptible to FCWP after VHT, and mature fruit were more prone to FB regardless of the phytosanitary VHT protocol. Fruit in relatively 'long' export supply chains had higher likelihood of expressing FB. Harvesting fruit at optimum maturity and / or sorting them after harvest for maturity can assure less IDs in 'B74' fruit. These findings contribute substantially to 'B74' mango supply chain sustainability. Further research into characterisation and quantification of other pre- and / or post-harvest factors potentially predisposing 'B74' fruit to IDs is still warranted towards eliminating them.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Publications included in this thesis

Chapter 3 integrated this conference paper:

Khanal, A., D. C. Joyce, M. A. Ullah, D. E. Irving, A. J. Macnish, P. A. Joyce, N. A. White, E.W. Hoffman, and R. I. Webb (2023) Fruit maturity and vapour heat treatment influence 'flesh cavity with white patches' disorder in Calypso^{TM} mango. 34th International horticultural congress. International Symposium on Postharvest Technologies to Reduce Food Losses, August 14-20. Acta Horticulture.

Submitted manuscripts included in this thesis

No manuscripts submitted for publication.

Other publications during candidature

Conference paper:

Ullah, M. A., Joyce, D. C., Joyce, P. A., White, N. A., Macnish, A. J. and Webb, R. I. (2023) Mineral nutrition and internal defects in vapour heat treated mango fruit. The 34th International horticultural congress, International Symposium on Plant Nutrition, Fertilization, Soil Management, August 14-20, 2022. Acta Horticulture (in press).

Magazine article:

Macnish, A., Joyce, D. C., Ullah, M. A., Khanal, A., and Dellis, P. (2021) Mango fruit quality for export supply chains. Australian Tree Crop, June/July 2021. Pp 26-27.

Book chapter:

Khanal, A., Mazhar, M.S., Weerahewa, H.L.D., Wyatt, P. and Joyce, D. (2023) Postharvest Technology and Quarantine Treatments (Chapter 15). In R. E. Litz (Ed.), The Mango Botany, Production and uses (3rd edition ed). CABI. (Submitted)

Contributions by others to the thesis

1. Professor Daryl C. Joyce (Thesis Principal Advisor, UQ Honorary Professor): Conception and design of the project; advising on individual experiments; critically revised the draft.
2. Donald Irving (Thesis advisor, former UQ Professor): advising on individual experiments; critically revised the draft.
3. Neil White (Thesis advisor, Department of Agriculture and Fisheries): advising on individual experiments; critically revised the draft.
4. Lynn Hoffman (Thesis advisor, UQ Lecturer): advising on individual experiments; critically revised the draft.
5. Richard Webb (Thesis advisor; UQ Adjunct Senior Lecturer): advising on individual experiments; critically revised the draft.
6. Priya Joyce (Former Principal Investigator at Sugar Research Australia): Critically advised on individual experiments, revised the manuscript.
7. Andrew Macnish (Senior Horticulturist, Department of Agriculture and Fisheries): Critically advised on individual experiments.
8. Vincent Mellor (UQ Biometrician): Advised on research design, data analysis, and interpretation of the results.
9. Lawrence Smith (Senior Technical Officer, Department of Agriculture and Fisheries): Contributing to conduct CA and respiration trials.
10. Hung Duong (Research Horticulturist, Department of Agriculture and Fisheries): Contributing to conduct CA and respiration trials.
11. Khamla Mott: Contributed to revising of draft and assist in data collection.
12. Darcy Holmes: Contributed to providing the fruit samples and vapour heat treatment.

Statement of parts of the thesis submitted to qualify for the award of another degree

No works submitted towards another degree have been included in this thesis.

Research Involving Human or Animal Subjects

Grower and processor survey was conducted in this thesis. Ethics approval was obtained from the UQ Human Ethics Unit. A copy of ethics approval letter is attached in the Appendix 8.

Ethics approval number: 2020001261

Name of approving committees: University of Queensland Science, Low and Negligible Risk Ethics Sub-Committee

Acknowledgements

I would like to express my deepest gratitude to my principal supervisor, Daryl Joyce, for his unwavering belief in me and guidance throughout my entire PhD journey. His nurturing support has been instrumental in my growth as a researcher. Furthermore, I extend my appreciation to my co-supervisors, Donald Irving, Neil White, Lynn Hoffman, and Richard Webb, for their invaluable guidance, support, and encouragement. Their expertise and unwavering belief in my abilities have played a pivotal role in the successful completion of this thesis.

I am sincerely grateful to the Cooperative Research Centre for Developing Northern Australia for their financial support in this project, as well as to Perfection Fresh Australia, the Department of Agriculture and Fisheries, and The University of Queensland for their generous contributions. Their support has been crucial in providing the essential resources and infrastructure that enabled the successful execution of this research.

I am grateful to the 'B74' mango growers from Northern Territory, North Queensland, and Simpson farm for their collaboration and provision of mango samples, which were vital to the success of this research. Their contributions and willingness to share their knowledge and expertise are deeply appreciated. Additionally, I would like to thank Robert and Grantt from Tomra/Compac for their invaluable collaboration in this study.

I am sincerely grateful to Joyce, Lawarance, Khamla, Yiru, Matt, Saroj, Khem, Mukunda, Sandip, and Hom for their invaluable assistance in data collection during my research. I am deeply grateful for the guidance and support provided by Priya in research planning and thesis drafting. Additionally, I appreciate Gary’s substantial help in imaging mango samples using MRI and X-ray µCT. Furthermore, I am thankful for the statistical support from Vincent Mellor and the contributions of my colleague Asad to this research.

Finally, I would like to sincerely thank my family and friends for their unwavering support and encouragement throughout this journey. Their love, support, and understanding were instrumental in helping me overcome the challenges I faced during my PhD. Without their assistance, I would not have been able to complete this thesis, and I am profoundly grateful to them.

Last but not least, I would like to express my appreciation to all who have supported me directly or indirectly throughout my PhD journey. Their invaluable contributions played a crucial role in the successful completion of this thesis, and I am deeply grateful to them. I hope that this research advance knowledge and benefit the wider community.

Financial support

This research was jointly supported by the Cooperative Research Centre for Developing Northern Australia which is part of the Australian Government's Cooperative Research Centre Program (CRCP), Perfection Fresh Australia, and the Department of Agriculture and Fisheries and The University of Queensland. This research was also supported by an Australian Government Research Training Program Scholarship.

Keywords

Calypso™, dry matter percentage, fruit maturity, internal disorders, near-infrared spectroscopy, supply chains and vapour heat treatment.

Australian and New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code: 070605 Post Harvest Horticultural Technologies (inc. Transportation and Storage) 100%

Fields of Research (FoR) Classification

FoR Code: 0706 Horticultural Production 100%

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Chapter 1 General introduction

1.1 Research background

Mango (*Mangifera indica* L.) is often referred to as the 'king of the fruits' (Asrey *et al.*, 2013; Yadav and Pandey, 2016). It is an economically important fruit crop belonging to the Anacardiaceae (Joyce *et al.*, 1993; Bally, 2006; Tharanathan *et al.*, 2006; Yahia, 2011; Asrey *et al.*, 2013; Rungpichayapichet *et al.*, 2016). The fruit is one of the most consumed fruits around the world (Yahia, 2011; Okino-Delgado *et al.*, 2020). It is highly nutritious, containing carbohydrates, proteins, fats, minerals, and vitamins (Mukherjee and Litz, 2009; Izneid *et al.*, 2014).

Mango is a major fruit crop in tropical and subtropical regions (Mukherjee and Litz, 2009). Its distribution in the global market is expanding (Yadav and Singh, 2017). Mango ranked fifth in the world in terms of total fruit production (Gómez-Ollé *et al.*, 2023). It ranked second among tropical fruits, after banana (FAO, 2024). India leads mango production, accounting for approximately 50% (24.7 million tonnes) of global mango supply, followed by Indonesia, China, Pakistan, Mexico, and Brazil (FAO, 2024).

In the year ending June 2020, total mango production in Australia amounted to 72 thousand tonnes, valued at $185.2 million farm gate. Most of this production is centred in two states, the Northern Territory (NT; 51%) and Queensland (Qld; 45%) (Figure 1.1) (Hort Innovation, 2020). Of this production, 88% was for domestic fresh supply and valued at $218 million, and 7% was for fresh export, worth $26 million (Figure 1.2) (Hort Innovation, 2023).

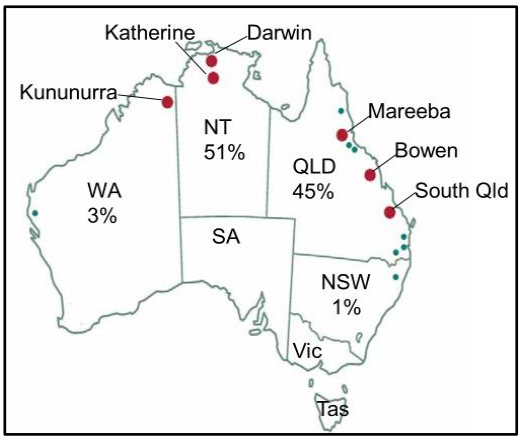


Figure 1.1: Fresh mango production in Australia by state and the major growing areas in each state. The proportion presented for each state represents share of total production. WA: Western Australia; NT: Northern Territory; QLD: Queensland; NSW: New South Wales; SA: South Australia; Vic: Victoria; and Tas: Tasmania. Source: (Hort Innovation, 2020).

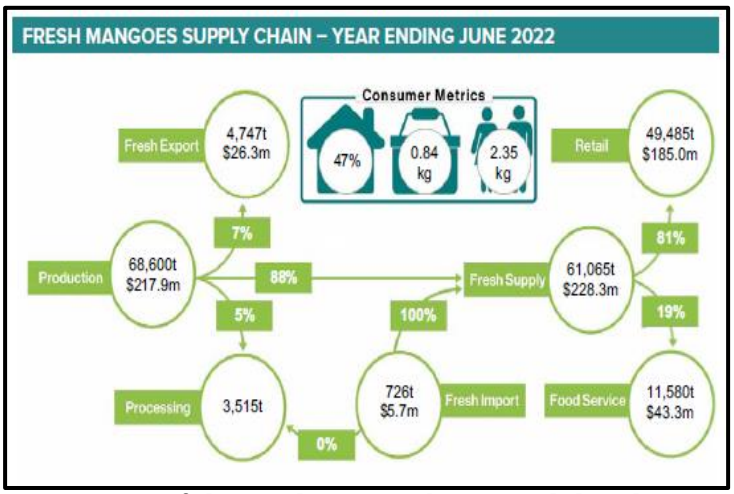


Figure 1.2: Representation of the production volumes and distribution of Australian mangoes in various sector. Source: (Hort Innovation, 2023).

Australia is a net exporter of fresh mangoes. The industry has demonstrated consistent growth over the years. The Australian industry exhibited year-on-year export growth, with volumes increasing by 67% from 2013 to 2020 (Figure 1.3). Annual growth rate was ~10%, with exception of two years disrupted by the global COVID-19 pandemic (Hort Innovation, 2022a). Increasing export volumes indicate that Australian mangoes are well-received by international consumers and suggest the possibility of expanding into new markets. The Australian mango industry aims to achieve growth by increasing the proportion of production exported from 9% to 20% (Russell, 2020). This increase is expected to lead to a growth of total production.

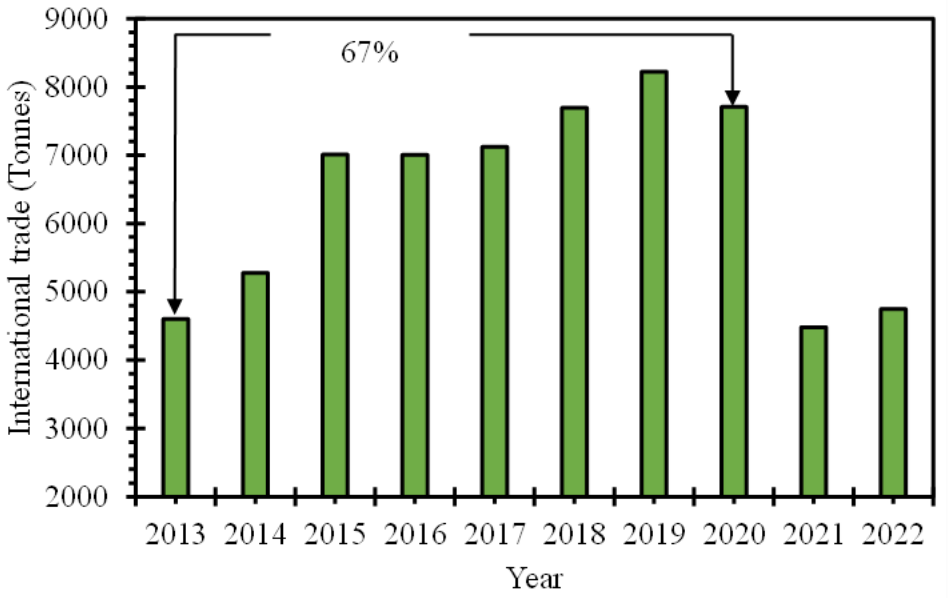


Figure 1.3: Growth in Australian mango exports from the year 2013 to 2022. Source: (Hort Innovation, 2023).

A range of mango varieties are grown in Australia. The fresh market is dominated by 'Kensington Pride' (KP), 'B74' (Calypso™), 'R2E2', and 'Honey Gold' (Figure 1.4). 'KP' is the most widely grown mango variety in Australia and representing 43% of fresh production. 'B74' is another popular variety. With yellow-orange skin and red blush, it accounts for 25% of fresh production (Hort Innovation, 2020). Its commercialization began in 1999 (Hofman, 2011) and rapidly became one of the most popular Australian mango variety. In addition to, it is favoured for flavour, freedom from fibre, and good retail shelf-life (Hofman *et al.*, 2010).

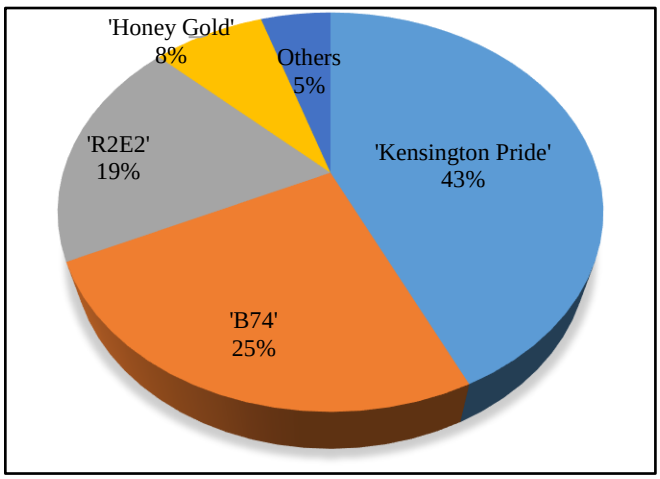


Figure 1.4: Mango production by varieties in Australia. (Hort Innovation, 2020).

However, variability and poor quality in mangoes associated with internal disorders (IDs) are obstacles to full value, competitiveness, and consumer confidence for Australian fresh mango supply chains (Hofman, 1995; Ledger, 1996). Post-harvest issues in 'B74' supply chains include flesh browning and flesh cavity with white patches (Figure 1.5) and have led to fruit rejection in the market, with negative economic consequences (Holmes, 2019a, 2019b). Causes and controls of IDs in 'B74' supply chains are poorly understood. It is therefore crucial to gain comprehensive understanding of contributing factors. Research-based recommendations are required to inform production of resistant fruit and to detect susceptible fruit during postharvest handling such that they can be selectively assigned to either relatively 'short' domestic or 'long' export chains.

The major export markets for Australian mangoes are East Asian countries, including China, Japan, Hong Kong, and South Korea. These account for 37% of total export (Hort Innovation, 2022a). Export markets have varying levels of access and phytosanitary treatment requirements (Hort Innovation, 2022b). The Australian mango industry has Japan, the USA, India, the Netherlands, and China as priority markets. Of these, only India and the Netherlands are non-protocol markets (Hort Innovation, 2022b). For export to the USA, it is mandatory that fruit to undergo gamma irradiation treatment with a minimum absorbed dose of 300 Gy (Hort Innovation, 2022b). Vapour heat treatment (VHT) is required for China and Japan and involves heating the fruit core to a minimum of 47 °C for 15 mins at saturated hot vapour (Hort Innovation, 2022a). Phytosanitary market-access treatments can have additionally adverse impact on quality.

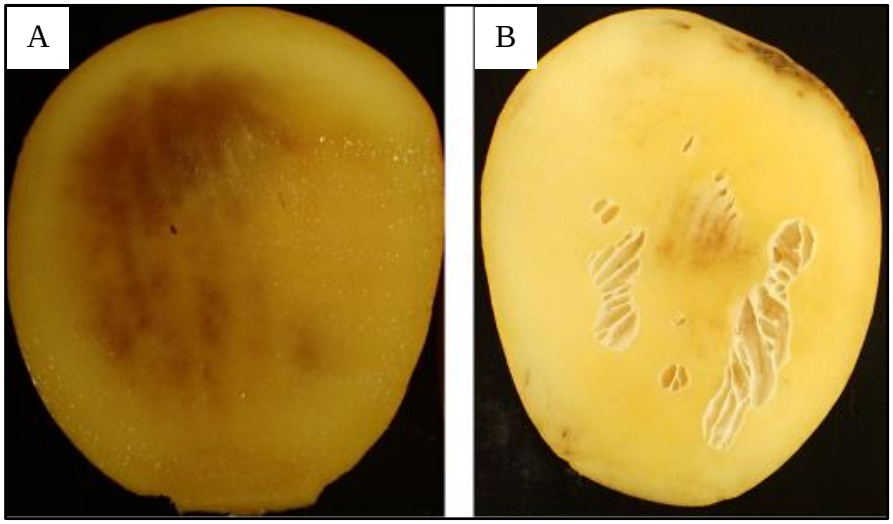


Figure 1.5: Symptoms of internal disorders (IDs) in 'B74' mango fruit; Flesh browning (A); and Flesh cavity with white patches (B).

The risk potential associated with exporting mangoes post-VHT is relatively high due to the prospective impact of phytosanitary treatment on fruit quality (Hort Innovation, 2022b). Research shows that VHT induces IDs in mangoes (Esguerra *et al.,* 1990; Joyce *et al.,* 1993) that include impaired starch degradation, internal cavities, and failure to 'colour up' (Figure 1.5 B) (Esguerra *et al.,* 1990; Joyce *et al.,* 1993; Jha *et al.,* 2010). In a study by Esguerra *et al.* (1990) VHT fruit showed 23 - 70% internal breakdown compared to 0 - 7% in untreated control fruit. Esguerrra and Lizada (1990) reported 18 - 58% of fruit affected by VHT versus only 0 - 11% in control fruit. Mitcham and McDonald (1993) reported that VHT fruit had 75 - 100% cavity and internal breakdown compared with 25% in untreated control fruit. Cavity- and internal breakdown symptoms in control fruit similar in appearance to VHT-induced damage may be attributable to exposure to high preharvest temperatures (>50 °C) (Katrodia and Sheth, 1988; Wainwright and Burbage, 1989).

Specific factors that contribute to the sensitivity of fruit to VHT have not yet been well characterised. Factors including variety, fruit size, preharvest variables, harvest maturity, and postharvest treatments, including temperature conditioning and phytosanitary treatment duration are considered to affect sensitivity to VHT in mangoes (Jacobi *et al.,* 2001; Anwar and Malik, 2007). Of these, harvest maturity is likely a critical contributing factor (Anwar and Malik, 2007). Previous studies indicate that immature fruit display susceptibility to VHT-induced cavities with starchy lesions (white hard tissue within mesocarp formed by localised disruption of the starch) while over-mature fruit display flesh browning (Esguerra *et al.,* 1990; Jacobi *et al.,* 2001; Brecht *et al.,* 2020). It is common to find mangoes of different maturities in one consignment (Ainsworth, 2016). Hence, sorting fruit for maturity at harvest and sending only the most tolerant maturity fruit for VHT could prospectively reduce IDs in supply chains and thereby maintain product reputation and market confidence.

Consumer's decision to buy fresh produce typically depends upon external appearance, such as colour, size, and absence of defects (Opara and Pathare, 2014). On the other hand, internal quality is a driver of consumer satisfaction and repeat purchase (Chen and Opara, 2013a, 2013b; Magwaza and Opara, 2015). However, detection of IDs in fruit is challenging as they are not visible externally and symptoms may not appear immediately after treatment, but only at the ripe stage (Esguerrra and Lizada, 1990).

Increasing demand for high quality fruit in the market warrants development of efficient, feasible, cost-effective, and accurate methods for prediction and / or detection of

internal quality (Du and Sun, 2004; Chayaprasert and Stroshine, 2005; Jha *et al.,* 2010; Arendse *et al.,* 2016a; Rungpichayapichet *et al.,* 2016; Suchanek *et al.,* 2017; Arendse *et al.,* 2018; Gabriëls *et al.,* 2020; Huang *et al.,* 2020; Mogollón *et al.,* 2020). Although destructive practices are commonly used to assess internal fruit quality (Arendse *et al.,* 2016b), non-destructive methods of quality evaluation are preferred. Non-destructive methods ideally enable measurement and analysis of individual fruit (Nicolai *et al.,* 2007; Huang *et al.,* 2020), permit repeated measurements of the same item over time, reduce waste (Nicolai *et al.,* 2007), and help to ensure delivery of consistently high-quality fruit to market (Al-Haq and Sugiyama, 2004).

Several non-destructive techniques have been employed for quality determination in fresh produce. Techniques suitable for ID detection include magnetic resonance imaging (MRI), X-ray and computed tomography (X-ray CT), visible and near-infrared spectroscopy (Vis-NIRS), hyperspectral and multispectral imaging, and acoustic techniques (Jha *et al.,* 2010). Among these, Vis-NIRS has been extensively employed to predict and assess various quality attributes of fresh produce, including mangoes. However, its use for predicting or detecting the IDs in mangoes has not been extensively studied (Jha *et al.,* 2010; Gabriëls *et al.,* 2020). Limited research has been conducted on detecting external and internal defects in ripe mangoes (Rivera *et al.,* 2014; Gabriëls *et al.,* 2020). There is little information as to using in-line Vis-NIRS for predicting IDs in mango fruit (Mogollón *et al.,* 2020). Against this background, this study was designed with the following aim and objectives.

1.2 Aim and objectives

The aim was to reduce internal disorders in ‘B74’ mango fruit for export supply chains.

The objectives included:

- Identify contributing factors to IDs.
- Assess maturity effects regarding expression of IDs.
- Seek non-destructive revelation of physiochemical changes during handling and ripening.
- Explore efficacy of in-line Vis-NIRS to predict maturity, TSS and / or IDs.

1.3 Approach

Given limited understanding of factors associated with the IDs of 'B74' mangoes, a comprehensive literature review was conducted for factors associated with IDs in fruits (Figure 1.6). Many factors were implicated in different fruits under varying conditions. Identification of a single factor associated with IDs in 'B74' supply chains was considered unrealistic. Therefore, the study adopted four approaches: an epidemiological study, controlled experiments, physiochemical assessment, and technology evaluation. Epidemiology studies examined putative associations among production and postharvest handling factors and IDs. It involved grower and handler surveys and incidence and severity monitoring of IDs in supply chains from different sources. The literature review and surveys collectively suggested that fruit maturity was pivotally linked with IDs in 'B74' mango fruit. Accordingly, the putative association of fruit maturity with IDs was evaluated in controlled experiments over two fruiting seasons. Physiochemical assessment of differing maturity fruit was conducted at serial stages of ripening, including using MRI and X-ray µCT techniques. In-line Vis-NIR technology testing was performed with providers to evaluate efficacy in predicting the internal quality for 'B74' mango fruit.

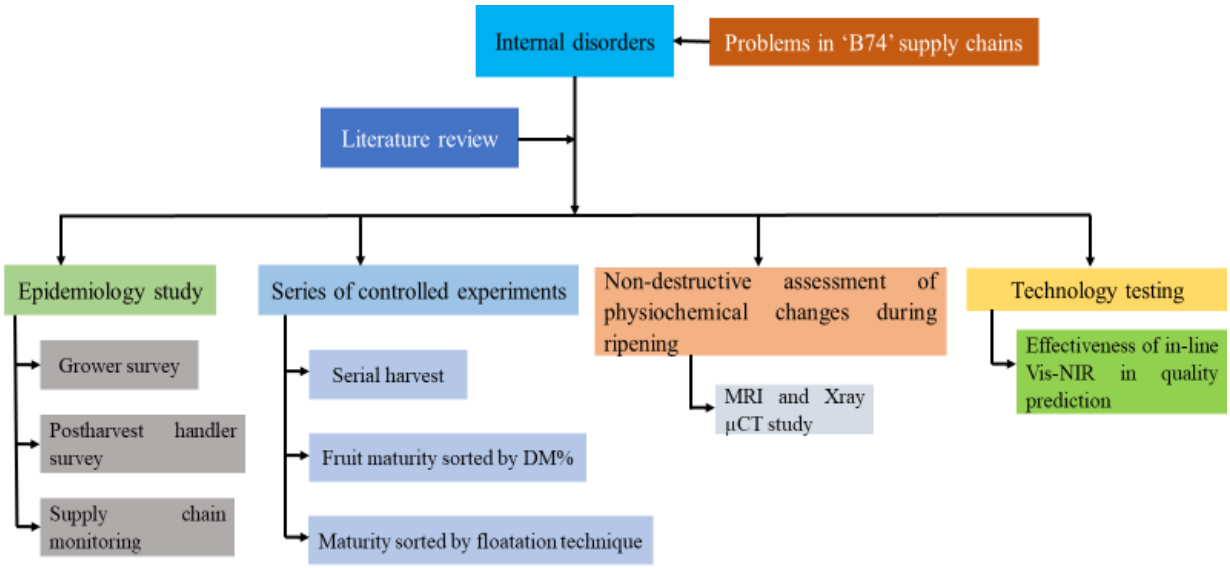


Figure 1.6: Study approach towards addressing internal disorders (IDs) of 'B74' mango fruit destined for export.

1.4 Thesis structure

Chapter 1 presents an introduction to the research, including an overview of background, problems, rationale, and objectives of the study. Chapter 2 presents in-depth review of pre- and post-harvest factors that affect fruit quality, with a focus on IDs and Vis-

NIR technology for their detection and prediction. Chapter 3 addresses factors linked with expression of IDs in ‘B74’ mango fruit along the supply chain. Chapter 4 focuses on fruit maturity and incidence and severity of IDs. Chapter 5 explores physiochemical changes over ripening for differentially mature fruit using advanced imaging. Chapter 6 examines efficacy of commercial in-line Vis-NIR technology for non-destructive prediction of internal quality and disorders of ‘B74’ mango. Finally, Chapter 7 offers discussion of research findings and conclusions from the study.

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Chapter 2 Factors associated with and non-destructive technologies to predict or detect internal disorders: A review

This chapter reviews literature on internal disorders (IDs), factors associated with, and non-destructive technologies to predict or detect these for mango fruit. Navigating the extensive and rapidly growing publication poses challenges for a comprehensive literature review. To address this, Google Scholar was employed to selectively search for and analyse relevant studies on individual topics. Additionally, project reports were consulted to supplement the review process. This focused strategy ensured a meaningful exploration within the constraints of the scholarly landscape.

2.1 Internal disorders

IDs are widespread fruit quality problems across mango cultivars (Arpaia, 1994; Torres *et al.,* 2004a; Bally, 2006; Ma *et al.,* 2018; Mogollón *et al.,* 2020). They are non-pathogenic disorders given either specific names or described broadly as 'internal flesh breakdown', 'internal breakdown' or 'IDs' that predominantly affect the fruit mesocarp (Bally, 2006). Underlying IDs in mango fruit forms of premature and / or uneven mesocarp ripening (Raymond *et al.,* 1998a).

Though symptoms of disorders express differently, many have been related to nutrient imbalances, including between calcium (Ca) and nitrogen (N). Preharvest factors which affect calcium accumulation in fruit are likely to result in calcium-related physiological disorders (Ferguson, 1984). Poor cell integrity of fruit is associated with low calcium in the flesh and manifests in various forms (Bally, 2006). The absence of external symptoms makes it difficult to detect IDs at harvest or during ripening. Typically, they are discerned upon cutting fruit open (Bally, 2006; Jha *et al.,* 2010; Ma *et al.,* 2018). IDs at best fetch low fruit price in the market because class 1 mango fruit may be graded into class 2 or 3 after quality check or inspection or at worst render them unfit for marketing / consumption (Katrodia, 1985). Therefore, IDs are problematic for fruit marketability and consumer satisfaction. Market quality specifications of 'B74' mango fruit and USDA quality check and inspection protocol is mentioned in Appendix 47.

Commonly reported IDs of mango include jelly seed, soft nose, stem end cavity, flesh browning, and spongy tissue (Arpaia, 1994; Bally, 2006).

Jelly seed is associated with ripening (Oosthuyse, 1993) and primarily observed in fully ripe fruit. It is characterised by watery, translucent, and jelly-like flesh, and ultimately complete mesocarp disintegration around the seed imparting over-ripe appearance (Figure 2.1; left) (Mogollón *et al.*, 2020). Affected flesh often also has off-odour and flavour (Hofman *et al.*, 2010).

Soft nose is somewhat like jelly seed. Flesh softening starts from the distal stigma or nose end of the fruit (Figure 2.1; middle) (Oosthuyse, 1993). In severe cases, flesh around the seed forms greyish-black spongy mass (Young, 1957; Young and Miner, 1961). Also, peel around the nose turns yellow before the rest of the fruit's outer appearance (Hofman *et al.*, 2010). In some instances, over-ripe tissue is bitter in taste (Bally, 2006).

Stem end cavity occurs between the stem end and the seed. Initial symptoms manifest as watery patches in the flesh, often accompanied by discoloured strands. As the disorder progresses, flesh collapses, leaving a cavity (Figure 2.1; right). Additionally, the flesh surrounding the cavity may turn grey-brown in colour (Hofman *et al.*, 2010).



Figure 2.1: Jelly seed (Hofman *et al.*, 2010), soft nose (Lin *et al.*, 2013), and stem end cavity (left to right) symptoms (Hofman *et al.*, 2010).

Spongy tissue is observed in fully ripe fruit and is characterised by sponge-like tissue between the pulp and seed (Katrodia, 1985). Affected tissue shows soft, leathery or spongy texture, possibly with air pockets and / or off-flavour (Subramanyam *et al.*, 1971). It is linked to unhydrolysed starch due to pre- or post-harvest heat exposure (Katrodia and Rane, 1979). Causes, mechanisms, and controls are still not fully understood. It was reported to be related to seed germination within the fruit (Ram *et al.*, 2020). The disorder particularly afflicts the popular Indian variety 'Alphonso' (Katrodia, 1985). Harvest at three-fourth maturity and low

temperature storing can reduce its incidence (Ram *et al.*, 2020). Spongy tissue symptoms resemble those induced by heat disinfestation methods (Chapter 1; Figure 1.5 B).

Flesh browning (FB) is diffuse brown discolouration in the fruit flesh (Chapter 1; Figure 1.5 A) (Mogollón *et al.*, 2020). Underlying causes and mechanisms of this disorder are poorly understood (Mellidou *et al.*, 2014; Gabriëls *et al.*, 2020; Mogollón *et al.*, 2020). Typically, FB is associated with O_2 starvation (Espín and Tomas-Barberan, 2001; Mellidou *et al.*, 2014). Efficient control methods are lacking, and so postharvest losses can be significant (Mogollón *et al.*, 2020). Mango FB occurs during over-ripeness stage (Gabriëls *et al.*, 2020).

Ullah et al (2023; Unpublished) conducted a systematic review on factors linked to mango fruit IDs, with the summarized outcomes presented in Figure 2.2.

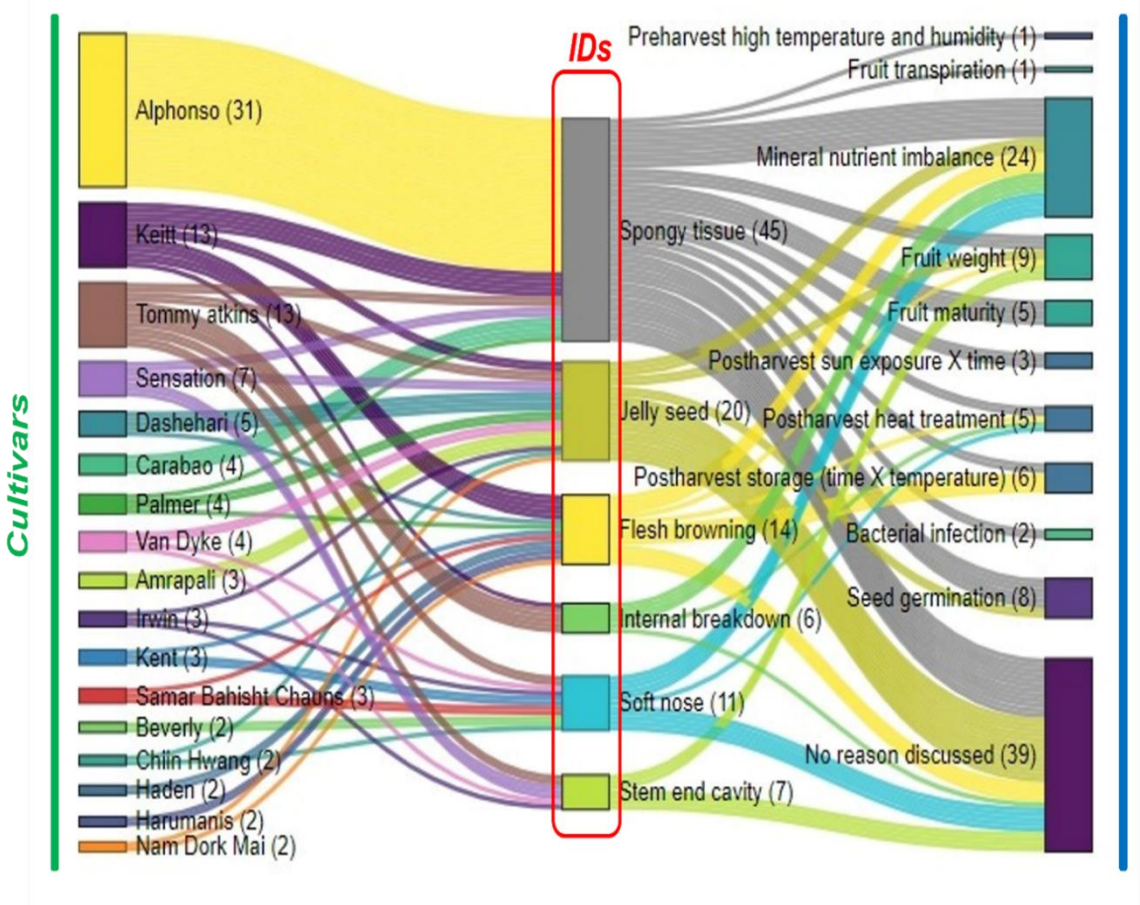


Figure 2.2: Exploration of major internal disorders (IDs) in mango fruit and associated factors across diverse varieties. Adapted from Ullah et al. (2023; Unpublished).

2.2 Effectors of internal disorders of fruit

Fruit susceptibility to IDs is associated to various preharvest, harvest and postharvest factors. Figure 2.5 illustrates the factors linked with IDs development in different fruits.

2.2.1 Preharvest factors

A range of genetic, environmental, and agronomic practices, including plant nutrition, affect fruit quality and physiological disorders (Bower and Cutting, 1987; Arpaia, 1994; Crisostol *et al.,* 1997; Whangchai *et al.,* 2001; Bally, 2006; Tharanathan *et al.,* 2006; Joas *et al.,* 2012; Rungpichayapichet *et al.,* 2016). These factors impact fruit growth and development (Arpaia, 1994; Arpaia *et al.,* 2004) by altering water and dry matter accumulation and biochemical and elemental constituents (Léchaudel and Joas, 2007). Minimising preharvest stress is important for good fruit quality (Bower and Cutting, 1987). Key preharvest factors include, but are not limited to, environmental, plant nutrient (especially Ca and N), crop load, and water management (Bally, 2006).

Mango fruit exposed to higher temperature (>50 °C) / excessive convective heat fluxes from the soil / air can develop internal flesh breakdown (Katrodia and Sheth, 1988; Wainwright and Burbage, 1989). Mango fruit growing in hotter locations may be less sensitive to heat disinfestation (Joyce and Shorter, 1994). Rainfall around harvest can make mango fruit more sensitive to disinfestation heat (Jacobi *et al.,* 2001a). 'Kensington Pride' mango harvested following prolonged heavy rainfall and subjected to vapour heat treatment (VHT) (46.5 °C core temperature, 10 mins) exhibited increased skin browning and internal starch layer (Jacobi and Wong, 1993).

Utilization of inputs that accelerate growth and maturation of fruit may force cell division and expansion that, in turn, increases susceptibility to abiotic stress, physiological disorders, and nutritional insufficiencies (Papadopoulos, 2020). High N in plants is associated with higher vegetative growth. Factors that lead to strong vegetative growth may increase Ca-related physiological disorders of fruit (Willingham *et al.,* 2004). Low Ca accumulation in fruit on high vigour plants reflects leaf transpiration dominance over fruit for Ca partitioning (Cull, 1991). Higher N levels increase fruit growth rate and Ca demand of fruit with faster growth rate cannot then be met. This can lead to calcium deficiency and increased Ca-related physiological disorders (Ferguson, 1984).

Ca is a key component of the cell wall middle lamella and needed to maintain cell integrity, cell wall strength in addition to an important role in membrane function (Hepler and Wayne, 1985; Poovaiah *et al.,* 1988; Hernández-Muñoz *et al.,* 2006) so as to mitigate storage disorders (Bangerth, 1979; Ferguson, 1984; Thorp *et al.,* 1997). Factors that interfere Ca accumulation of in fruit can cause IDs (Ferguson and Watkins, 1989). Ca deficiency cause internal flesh breakdown in mango (Raymond *et al.,* 1998b; Whangchai *et al.,* 2001; Torres *et al.,* 2004b; Bally, 2006; Ma *et al.,* 2018).

Deficit irrigation during early fruit growth and development affect Ca accumulation in fruit as it is predominantly transported and accumulated during this period (Ferguson and Watkins, 1989). Preharvest water stress increases fruit polyphenol oxidase levels, which are linked to mesocarp discolouration (Bower and Cutting, 1987; Bower, 1988). Capacity to withstand low O_2 and high CO_2 is low in water-stressed avocado fruit (Bower and Cutting, 1987; Bower, 1988).

Canopy management should prevent excessive competitive vegetative growth during fruit development (Arpaia *et al.,* 2004). Vegetative growth that competes with fruit growth, especially if it coincides with early fruit development, leads to Ca imbalance in the fruit and overall reduction of quality (Arpaia, 1994; Arpaia *et al.,* 2004). The key period for uptake of Ca is fruit set to stone hardening (Hofman and Whiley, 2010). Any competition for Ca at this stage will lead to low-Ca fruit prone to IDs.

Low leaf-to-fruit stimulates Ca accumulation in mango fruit and reduces disorders (Lechaudel *et al.,* 2005). Low leaf-to-fruit have high Ca:(K+Mg) (Simmons *et al.,* 1998a; Simmons *et al.,* 1998b). Relationship between crop load and physiological disorders, like watery-pulp breakdown in mango have been described by several researchers (Hofman *et al.,* 2002; Bally, 2006).

2.2.2 Harvest factors

Two key factors linked to IDs are time of harvest and harvest maturity.

2.2.2.1 Time of harvest

Harvest timing impacts postharvest fruit quality, with the coolest part of the day being optimal (Johnson and Hofman, 2009). ‘Honey Gold’ mango harvested at night or early morning

were relatively more resistant to under skin browning (USB) disorder compared to late morning or afternoon harvested fruit (San *et al.,* 2019).

2.2.2.2 Harvest maturity and physiological disorders

Being a climacteric fruit, mango is harvested in the hard-green mature stage after attaining physiological maturity but before the climacteric rise (Joyce *et al.,* 2002; Joas *et al.,* 2012; Anderson *et al.,* 2020). The severity of physiological disorders increases with advancing fruit maturity (Arpaia, 1994). Examples of such disorders include watery pulp breakdown (Bally, 2006), stem end cavity (Hofman and Whiley, 2010), and jelly seed in mango fruit (Yahia, 1998; Yahia, 2005a; Hofman and Whiley, 2010). Similarly, late-harvested fruit exhibited higher incidence of lenticel spotting, skin browning, body rot, stem-end rot, and dendritic spots compared to early-harvested mango fruit (Hofman, 2011). In contrast, as fruit maturity increased, the sensitivity of fruit to disinfestation heat treatment (VHT / HWT) induced disorders, such as cavities and starchy layers, decreased (Esguerra *et al.,* 1990; Jacobi *et al,* 2001a; Jacobi *et al.,* 2001b; Brecht *et al.,* 2020). Similarly, less mature mango fruit were more prone to chilling injury (CI) (Brecht *et al.,* 2020).

Therefore, harvesting at optimum maturity helps to minimise IDs in mango fruit. Hence, there is a need to establish an objective harvest index that can accurately classifies fruit of different maturity. Among various maturity indices, for mango, dry matter content (represented as a percentage of dry weight to fresh weight; DMC) is considered a reliable and objective indicator of maturity (Carvalho *et al.,* 2014; Anderson *et al.,* 2017; Kinhal, 2019; Anderson *et al.,* 2020), capable of accurately classifying fruit maturity and predict eating quality at ripe stage (Maldonado-Celis *et al.,* 2019; Hor *et al.,* 2020; Liguori *et al.,* 2020; de Freitas *et al.,* 2022).

2.2.2.3 Fruit maturity and eating quality

Eating quality is the minimum level of acceptable physio-chemical attributes in the fruit as would be perceived by the majority of consumers. Ripe eating quality of mango was determined based on TSS (Saranwong *et al.,* 2004; Subedi *et al.,* 2007). Total DMC is a measure of total starch at harvest (Subedi *et al.,* 2013). Starch is converted into TSS when ripe (Grechi and Normand, 2017; Kinhal, 2019). Anderson *et al.* (2017) reported a high linear correlation ($r^2 = 0.90$) between ripened TSS and fruit DMC at harvest. TSS was shown to be positively correlated to ripe stage eating quality in mango (Anderson *et al.,* 2020). Therefore,

mango DMC at harvest has a strong positive correlation with ripened fruit eating quality (Hor *et al.*, 2020).

Estimation of DMC at harvest, based on its strong relationship with eating quality, could be used as a maturity index for mango (Saranwong *et al.*, 2004). To achieve standardization of fruit quality with good eating quality for an export-based industry, maximum and minimum limits of DMC should be set (Carvalho *et al.*, 2014). However, a compromise of harvest maturity is required that maximises the eating quality and provides a sufficient harvesting window (Hofman and Whiley, 2010).

2.2.3 Postharvest factors

Postharvest factors significantly impact fruit quality and contribute to quality loss or variation after harvest. However, postharvest technologies cannot improve fruit quality, but only help to maintain it (Simmons, 1999; Hewett, 2006; Léchaudel and Joas, 2007). Therefore, it is critical to understand the key postharvest factors that influence fruit quality and physiological disorders. This section discusses these significant postharvest factors linked to IDs.

2.2.3.1 Precooling

Pre-cooling is a crucial step in cold chain management, involving quickly lowering the temperature of freshly harvested produce (Meurant *et al.*, 1999). Typical shelf-life of mango fruit is 15-20 days after harvest (Vazquez-Salinas and Lakshminarayana, 1985). Delaying mango cooling can result in faster ripening, shorter shelf-life, and poor quality (Yahia, 1998). However, Marques *et al.* (2014) and Marques *et al.* (2012) reported that fruit subjected to delayed cooling after harvest showed significantly low USB in 'Honey Gold' mango. This delay may enable the fruit to undergo partially ripening, and the subsequent increased in sugar content enhances their tolerance to such disorders. Similarly, Mohammed and Brecht (2002) also reported that partial ripen mango exhibited greater resistance to CI.

2.2.3.2 Storage temperature

Temperature plays a crucial role for maintaining mango quality after harvest (Al-Haq and Sugiyama, 2004; Léchaudel and Joas, 2007; Yahia, 2011; Brecht *et al.*, 2020). Mangoes stored in ambient temperature exhibited rapid softening and gradual decay (Couey, 1982; Al-Haq and Sugiyama, 2004; Ntsoane *et al.*, 2019b) due to enzyme-mediated cell wall degradation

that are accelerated by higher temperatures (Narain *et al.*, 1998). Therefore, low temperature storage is used to slow down fruit metabolism, delay ripening and reduce senescence (Sudhakar and Gopalakrishna, 2008).

Fruit stored outside of the recommended temperature range can result in failing to meet market quality standards (DAF, 2019). For example, mangoes stored at temperatures above 28 °C tend to remain green, while temperatures exceeding 33 °C for extended periods hinder ripening (Yahia, 2005b). Mango ripening occurs within the range of 15.5 - 30 °C, with the optimum temperature for achieving desirable colour, texture, and flavour being 18 - 22 °C (Brecht *et al.*, 2020). Temperature for better-quality mangoes during postharvest handling is described by Barker *et al.* (2007) (Figure 2.3).

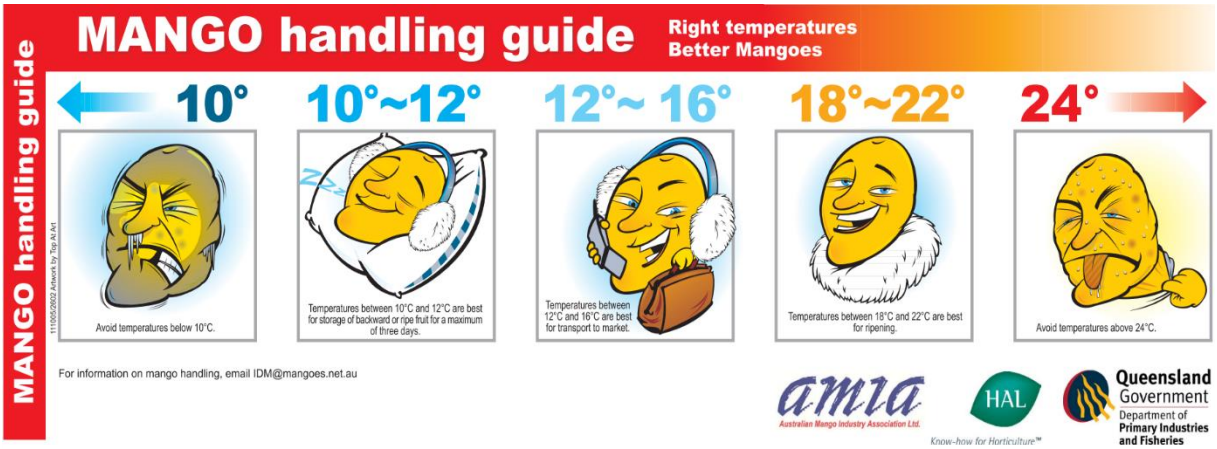


Figure 2.3: Recommended handling temperatures for better mangoes (Barker et al., 2007).

Low storage temperatures can cause CI (DAF 2019) and is characterised by surface pitting, greyish scald-like skin discolouration (Figure 2.4), and in severe cases, flesh browning (Brecht *et al.*, 2020). To prevent CI, mangoes should not be stored below 10 - 12.2 °C, depending on variety, maturity, and ripeness stage (National Mango Board n. d.). For example, 'Hongsawadee' demonstrated greater tolerance than 'Nam Dok Mai' and 'Chok Anan' when stored at 5 °C for 3 weeks (Siriphanich and Kunyamee, 2010). Immature mango fruit suffer damage even at temperatures above 12 °C and as the maturity progress, they can tolerate progressively lower temperature (Brecht *et al.*, 2020). Tree-ripe 'Keitt' and 'Tommy Atkins' mangoes exhibited less CI when stored at 5 °C for 2 weeks. However, mature green mangoes exposed to temperature below 10 °C can demonstrate severe CI (Singh *et al.*, 2013).



Figure 2.4: Symptoms of chilling injury as characterised by Shrunken, discoloured area (Brecht, 2020).

2.2.3.3 Storage atmosphere

The temperature recommendation for refrigerated storage of mangoes may not be adequately low to prevent ripening and senescence, particularly during sea freight. Controlled atmosphere (CA) in combination with low temperatures provides an effective alternative approach to prolong shelf-life, maintain fruit quality, and facilitate long-distance export, particularly for sea freight (Singh *et al.,* 2013). CA has notable effects on the physical, chemical, and physiological properties of mango fruit (Wei *et al.,* 2021). The optimal gas concentration for extended shipping or storage of mangoes are typically 3 - 5% O_2 and 5 - 10% CO_2 at 10 °C (Ntsoane *et al.,* 2019a). However, if the O_2 concentration drops below 2% (depending on cultivar, storage temperature, fruit maturity, and ripeness stage), anaerobic respiration occurs, resulting in off-flavours, and physiological disorders (Bender *et al.,* 2000; Ramayya *et al.,* 2012).

The gas diffusivity of tissues plays a crucial role in determining the tolerance of fresh produce to low O_2 and high CO_2 during postharvest storage (Ferguson *et al.*, 1999). The arrangement of cells and intercellular spaces within the fruit's microstructure creates specific pathways for gas diffusion (Herremans *et al.,* 2014a). During fruit maturation or ripening, cell-wall permeability changes (Cantre *et al.,* 2020) due to the activity of oxidative (Perotti *et al.,* 2014) and cell wall degrading enzymes (Sitrit and Bennett, 1998), leading to the loss of cell membrane integrity and the disruption of cellular compartmentalisation. These changes manifest as a decrease in pore size, increase in pore fragmentation and pore specific area (Cantre *et al.,* 2014) and can impact gas diffusion within the fruit flesh (Ho *et al.,* 2013; Ho *et*

al., 2014). Therefore, in general, more mature or ripe fruit are prone to O_2 starvation in the fruit flesh, especially in the inner mesocarp and cause FB (Espín and Tomas-Barberan, 2001; Mellidou *et al.,* 2014). These findings align with the study by Ferguson *et al.* (1999) on the causes of internal browning in apple.

2.2.3.4 Postharvest treatments

2.2.3.4.1 Vapour heat treatment (VHT)

Tephritid fruit flies pose a serious quarantine risk (Heather *et al.,* 1991). Freedom from infesting insects must be guaranteed to facilitate international trade. Postharvest heat disinfestation, by vapour heat, emerged as a viable non-chemical and internationally acceptable control method for fruit flies in the mango fruit (Joyce *et al.,* 1993; Jacobi *et al.,* 2001a). VHT involves the heating of fruit core with saturated hot vapour (47 °C for 15 mins) to disinfest all stages of fruit flies that may be present in fruit (Jacobi *et al.,* 2001a).

However, VHT can results in different types of serious IDs (Esguerra *et al.,* 1990; Joyce *et al.,* 1993) characterised by cavities with white, starchy, and tough lesions in mesocarp (Esguerra *et al.,* 1990; Jha *et al.,* 2010); Chapter 1; Figure 1.5 B). In extreme conditions, VHT can damage all the fruit due to an induced internal O_2 deficiency and results in undesirable fermented by-products (Esguerra *et al.,* 1990; Esguerrra and Lizada, 1990). VHT-induced internal breakdown is not visible externally and symptoms will remain hidden until the ripe stage (Esguerra *et al.,* 1990; Esguerrra and Lizada, 1990). VHT-treated 'Carabao' mango fruit showed 23 - 70% internal breakdown compared to 0 - 7% in control fruit (Esguerra *et al.,* 1990). Esguerrra and Lizada (1990) also reported 18 - 58% of 'Carabao' mango fruit were affected by VHT versus significantly lower incidence (0 - 11%) in control fruit. Mitcham and McDonald (1993) reported that VHT 'Tommy Atkins' mango fruit showed 75 - 100% cavity and internal breakdown versus 25% in control fruit. Cavity- and internal breakdown noted in control fruit, not exposed to VHT, might be due to the exposure to higher preharvest temperatures (Katrodia and Sheth, 1988; Wainwright and Burbage, 1989).

2.2.3.4.1.1 Fruit maturity and VHT response

Incidence and severity of VHT-induced IDs in mango are influenced by different factors viz., variety, temperature, and duration of treatment, fruit size, preharvest factors, and prior conditioning before heat treatment (Anwar and Malik, 2007; Jacobi *et al.,* 2001a). In addition to these factors, harvest maturity is generically important when applying VHT

(Esguerrra and Lizada, 1990; Anwar and Malik, 2007). Relatively less mature mango fruit are more prone to IDs following disinfestation heat treatment (Brecht *et al.,* 2020), whereas over-mature fruit were more prone to FB. For example, immature 'Carabao' mango fruit exhibited higher susceptibility to internal cavity and starchy lesions following VHT than did mature fruit (Esguerra *et al.,* 1990). Similarly, immature 'Kensington Pride' mango fruit were also more susceptible to internal cavity and starchy lesions following hot water treatment (HWT) than mature fruit (Jacobi *et al.,* 2001b).

VHT-induced flesh cavity and starchy lesions were observed near and around the seed (Chapter 1; Figure 1.5 B). The development of white starchy lesions during fruit ripening is linked to enzymes responsible for converting starch into sugar that may denature during heat treatment, resulting in disruption of the starch degradation process (Joyce *et al.,* 1993). In mature fruit, enzymes such as alpha-amylase are necessary for starch degradation and may already have been activated for starch hydrolysis prior to harvest or heat treatment. However, in immature fruit, activation of these enzymes might not have commenced before heat treatment that may inhibit their subsequent activity and result in the observed starchy layers.

More mature fruit are susceptible to FB due to anatomical differences rather than biochemical differences (Esguerra *et al.,* 1990; Crisosto, 1995; Jacobi *et al.,* 2001a). Diffusivity of respiratory gases in inner mesocarp is reduced with increasing fruit maturity and ripening (Crisosto, 1995) (refer Section 2.2.3.3). VHT promoted respiration rates, and consequently, further starved O_2 in the inner mesocarp. For example, CO_2 production accelerated by three-and-half-fold when the fruit were heated at 46 °C and five-fold, when heated to 48 °C. The rate of respiration remained elevated for 4 to 6 days following heat treatment (Mitcham and McDonald, 1993). CO_2 concentration in the inner mesocarp, immediately after the treatment of 46 °C and 48 °C, was found to be 9% and 13% (v/v), respectively compared to 1.5% (v/v) in control fruit. Similarly, the O_2 concentration in control fruit was 19% (v/v) compared to 11% and 6% (v/v) when fruit were heated to 46 °C and 48 °C, respectively (Mitcham and McDonald, 1993).

To reduce the VHT-induced IDs in mango fruit, approaches such as shortening the handling period, effective sorting of immature and over-mature fruit, implementing fast cooling techniques, applying temperature preconditioning, and supplementing O_2 in the chamber, could all be beneficial (Esguerra *et al.,* 1990). After VHT, fruit should be protected from further water loss by placing fruit under high relative humidity (>95%) (Brecht *et al.,* 2020).

2.2.3.4.2 Temperature conditioning

Temperature conditioning involves subjecting the product to a higher temperature than the recommended storage temperature for a specific duration. A wait period minimum of 24 h under typical ambient temperature or conditioning treatment prior to disinfestation heat treatment helps to reduce or eliminate physiological disorders in mango fruit (Klein and Lurie, 1992; Joyce *et al.,* 1993; Joyce and Shorter, 1994; Jacobi *et al.,* 1995; Jacobi *et al.,* 2001a; Brecht *et al.,* 2020). However, it was not sufficient to completely eliminate disorders (Joyce and Shorter, 1994). This time delay or conditioning might benefit less mature fruit (Brecht *et al.,* 2020). HWT-treated fruit stored at 13 °C between harvest and treatment became unmarketable while maintaining them at ambient temperatures before temperature resulted in acceptable levels of injury (Smith and Chin, 1989).

The physiological age of fruit can be enhanced through conditioning or a waiting period, and it plays a crucial role in increasing the fruit's tolerance to disinfestation heat treatment. According to Jacobi *et al.* (1995), fruit treated immediately after harvest exhibited higher starch levels and lower sucrose content, resulting in a more severe starch layer disorder after heat treatment. Sugars, on the other hand, have been found to play a significant role in protecting plant tissues, including fruit, against various physical stress such as high CO_2, desiccation, cold, and heat (Jacobi *et al.,* 2002).

Heat shock proteins (HSPs) were found in the conditioned fruit in the temperature range of 38 - 40 °C and their accumulation was correlated with increased heat tolerance (Jacobi *et al.,* 2001a). In avocados, HSPs gene expression and protein levels were found to increase in response to postharvest heat treatments, and pre-treatment at 38 °C was associated with enhanced fruit tolerance to potentially damaging heat treatments (e.g., 50 °C) (Paull and Chen, 1990; Woolf *et al.,* 1995; Florissen *et al.,* 1996; Woolf and Lay-Yee, 1997).

2.2.3.4.3 Irradiation

Gamma irradiation is the market protocol treatment for Australian mangoes exported to certain countries, including New Zealand and the USA (Ainsworth, 2015; Marques *et al.,* 2022). A minimum dose of 400 Gy is required to sterilise Queensland fruit fly and is considered a standard treatment for most insects (Ainsworth, 2015).

Fresh fruits and vegetables are metabolically active and thus sensitive to ionising radiation (Sivakumar *et al.,* 2011). The effectiveness of irradiation on mango fruit quality

influenced by cultivar, irradiation dose, and fruit maturity (Sivakumar *et al.*, 2011; Sultana *et al.*, 2021).

Mango cultivars respond differently to irradiation doses (Thomas and Moy, 1986). 'B74' was more susceptible, while 'Kensington Pride' and 'R2E2' exhibited moderate sensitivity, and 'Honey Gold' was relatively more tolerant. 'Keitt' mangoes develop internal browning after 19 days of storage when exposed to 600 Gy or higher radiation, whereas 'Kent' and 'Ataulfo' varieties can withstand up to 860 Gy (Anynomous, n. d.).

The fruit's physiological stage or maturity at the time of irradiation significantly influences ripe fruit quality (Thomas and Moy, 1986; Boag *et al.*, 1990; Marques *et al.*, 2022). Less mature fruit is more affected by irradiation treatment, while partially ripe fruit in the climacteric phase is least affected (Boag *et al.*, 1990; Sultana *et al.*, 2021). For example, irradiating ripening 'B74' fruit (~70% yellow skin) with 300 - 800 Gy resulted in 74 - 100% marketable fruit at the ripe stage, versus 5 - 59% for green mature fruit (~10% yellow skin) (Marques *et al.*, 2022). Fruit treated at colour stage 2 - 3 incurred less irradiation damage, such as lenticel spotting and skin browning, and retained their green colour for an extended period compared to fruit treated at the green mature stage (Hofman *et al.*, 2014).

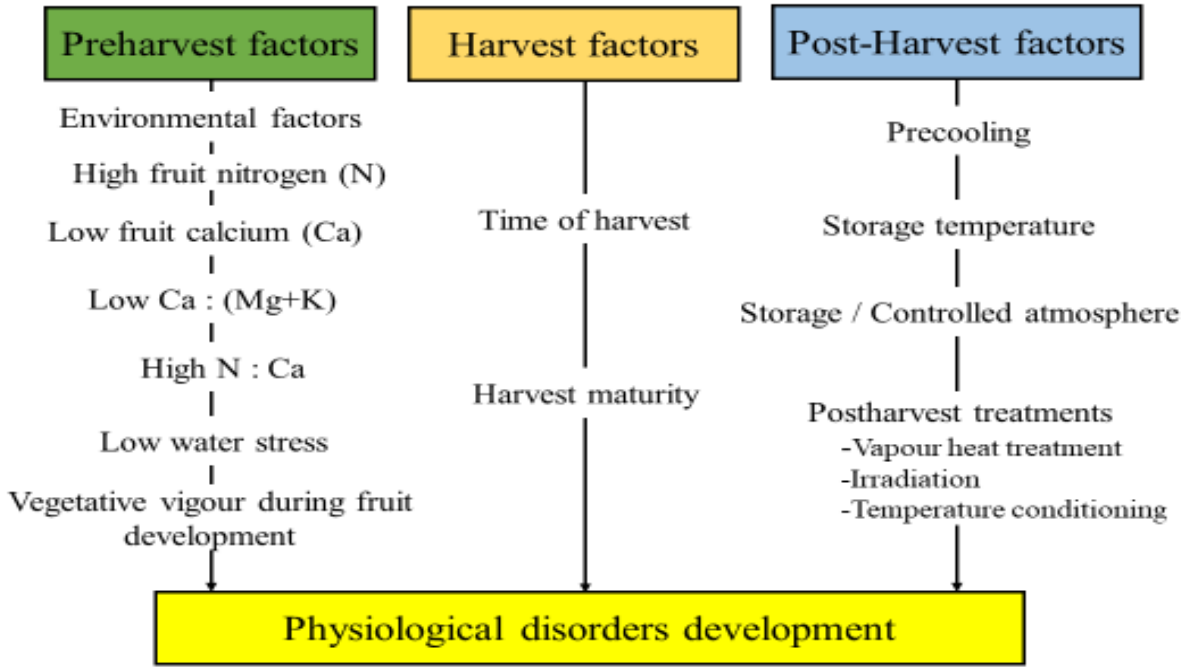


Figure 2.5: Illustration of preharvest and postharvest factors associated with the fruits internal disorders (IDs) development.

2.3 Non-destructive technologies for internal quality prediction / detection

To meet the demand for high-quality fruit in the market, efficient, accurate, cost-effective, feasible, and non-destructive methods to detect and predict internal quality is imperative (Du and Sun, 2004; Chayaprasert and Stroshine, 2005; Jha *et al.,* 2010; Arendse *et al.,* 2016a; Rungpichayapichet *et al.,* 2016; Suchanek *et al.,* 2017; Arendse *et al.,* 2018; Gabriëls *et al.,* 2020; Huang *et al.,* 2020; Mogollón *et al.,* 2020). Destructive practices have been commonly used to evaluate internal fruit quality (Arendse *et al.,* 2016b), non-destructive methods are now preferred because non-destructive methods enable measurement and analysis of individual fruit (Nicolai *et al.,* 2007; Huang *et al.,* 2020), permit repeated measurements over time, reduce waste (Nicolai *et al.,* 2007), and help to ensure delivery of consistently high quality fruit to market (Al-Haq and Sugiyama, 2004).

Various non-destructive techniques viz., visible and near-infrared spectroscopy (Vis-NIRS) (Appendix 1), hyperspectral and multispectral imaging (Appendix 2), X-ray and computed tomography (X-ray CT) (Appendix 3), magnetic resonance imaging (MRI) (Appendix 4), and acoustic techniques (Appendix 5) have been used for predicting fresh produce quality. The advantages and disadvantages of the above-mentioned techniques are described in the Table 2.1. Among these, Vis-NIRS has been extensively used techniques for quality attributes of fruits and vegetables (Appendix 1), including mangoes (Jha *et al.,* 2010).

2.3.1 Visible and near-infrared spectroscopy

The electromagnetic spectrum (400 - 2500 nm) is divided into two regions: Vis (400 - 750 nm) and NIR (750 - 2500 nm) (Arendse *et al.,* 2018; Walsh *et al.,* 2020a). Within NIR, the range of 750 - 1100 nm is referred to as short wavelength NIR or the Herschel region, while the range of 1100 - 2500 nm is known as the NIR proper region (Walsh *et al.,* 2020a). Utilising Vis-NIRS within the 400 - 1000 nm wavelengths is emerging as a valuable non-destructive technique for assessing the internal quality of mango fruit (Gabriëls *et al.,* 2020).

Vis-NIRS has been gaining interest for rapid, safe, sensitive, and non-destructive quality assessment of agro-products because it can access physical (McGlone and Kawano 1998; Penchaiya *et al.,* 2009; Kafle *et al.,* 2016; Escribano *et al.,* 2017; Xu *et al.,* 2019) and chemical properties linked to fruit quality (Moghimi *et al.,* 2011; Mishra *et al.,* 2017; Gabriëls *et al.,* 2020). Vis-NIRS is widely recognised as a prominent techniques for fruit quality evaluation (Butz *et al.,* 2005; Nicolai *et al.,* 2007; Izneid *et al.,* 2014).

Vis-NIRS assess absorbed light related to the vibration of molecular bonds, particularly O-H and C-H bonds (Kawano, 1994; Golic *et al.,* 2003), providing estimates of water and storage reserves in fruit (Walsh *et al.,* 2020a). This method offers insights into molecules abundant in hydrogen atoms, such as water, alcohol, amines, and compounds containing OH-, CH-, and NH- groups (Nicolaï *et al.,* 2014a; Arendse *et al.,* 2017).

An important consideration in using NIR is optical path length and density of the fruit as they can vary due to the differences in fruit size, thickness of rind and shape (Krivoshiev *et al.,* 2000; Magwaza *et al.,* 2012; de Oliveira *et al.,* 2014). Due to low penetration depth of Vis-NIR, physiological disorders and internal quality detection is limited in thick rind fruits (Arendse *et al.,* 2016b, 2016a, 2018) and reducing measurement accuracy (Nicolai *et al.,* 2007). Penetration depth is determined by the wavelength of light, such as 4 mm in the 700 - 900 nm range and 2 - 3 mm in the 900 - 1900 nm range for Jonagold apples (Lammertyn *et al.,* 2000) and is influenced by the tissue water absorption profile. In fresh fruits with higher water concentration, sufficient light penetration beyond the 200 - 1200 nm range is not achievable (Lammertyn *et al.,* 2000).

The application of Vis-NIRS in postharvest analysis of fresh produce is well-established (Walsh *et al.,* 2020b) and has been extensively used for predicting various quality attributes in different fruit and vegetables (Appendix 1). However, use of Vis-NIRS in predicting or determining the IDs of mangoes is relatively unexplored (Gabriëls *et al.,* 2020).

2.3.2 X-ray computed tomography (X-ray CT)

The use of X-ray CT has become one of the well-established and potential non-destructive techniques for studying the internal and external morphological characteristics and defects (Magwaza and Opara 2014; Arendse *et al.,* 2016b). It is a powerful tool used for non-destructive assessment for the internal disorder of fruits owing to its speed and penetration (Huang *et al.,* 2020).

X-ray CT measures variation in material density of the tissue (Zwiggelaar *et al.,* 1996; Butz *et al.,* 2005). X-ray attenuation for a particular material is approximately proportional to its density, sample thickness, absorption coefficient, and wavelength (Guelpa *et al.,* 2015; Arendse *et al.,* 2016b). Healthy and disorder tissue differs in the density thus producing distinct images which help to differentiate fruit internal disorders (Nicolaï *et al.,* 2014a; Arendse *et al.,* 2016a). X-ray CT number is the standard unit used for measuring X-ray absorption

characteristics. CT numbers are based on linear X-ray absorption coefficients and, in general, is expressed by brightness data in an image (Arendse *et al.,* 2016b). Different authors reported the use of X-ray in determining the quality of fruits and vegetables which is mentioned in Appendix 3. These studies suggest the potentiality of X-ray CT in determining the internal quality of fruits and vegetables.

2.3.3 Magnetic resonance imaging (MRI)

MRI is based on interaction of electromagnetic radiation in the radiofrequency range of the electromagnetic spectrum with an object (Nicolaï *et al.,* 2014b; Patel *et al.,* 2015), which allows non-destructive measurement of the spatial distribution of proton density and relaxation parameters in the sample by monitoring the proton dynamics (Nicolaï *et al.,* 2014a; Patel *et al.,* 2015). It offers a means of non-destructively examining internal defects (Lammertyn *et al.,* 2003b), visualize morphological changes and water density and mobility in intact plant tissue (Defraeye *et al.,* 2013; Suchanek *et al.,* 2017) and understanding of ripening related physiological processes (Butz *et al.,* 2005). MRI has been successfully applied to investigate the quality and IDs of various fruits and vegetables (Appendix 4). These studies suggest that MRI can be used to detect different quality parameters of fruits and vegetables.

To explore the properties of food materials MRI makes the use of the inherent magnetic properties that some atomic nuclei posses (Barreiro *et al.,* 2000; Butz *et al.,* 2005; Patel *et al.,* 2015), for instance, ^{1}H, ^{13}C, ^{31}P, ^{23}Na, a magnetic moment and can absorb resonance energy when placed in a strong magnetic field and irradiated with a proper radio frequency (Butz *et al.,* 2005; Patel *et al.,* 2015).

^{1}H MRI is the most straightforward, accessible and sensitive MRI probe for highly resolved spatial information concerning the distribution and magnetic environment of water in biological tissues (Clark *et al.,* 1997). Due to its sensitive nature, it can provide visualization of even minute localized morphological changes in intact plant tissues (Zemah *et al.,* 1999). Moreover, MRI has been used to quantitatively assess and study physiological changes like firmness and solubility of solids in fruit during maturation and storage as a function of the relaxation times (Andaur *et al.,* 2004).

Changes during ripening like starch hydrolysis to sugar, cell wall degradation, and loss of membrane integrity (Mitra and Baldwin, 1997) may influence the distribution and activity of protons (water) in mango fruit mesocarp (Létal *et al.,* 2003). This in turn modifies the

relaxation times of protons in water, which can be used as contrast parameters in the MR images enabling to observe even small changes of the fruit flesh composition, not visible with other methods (Suchanek *et al.*, 2017).

Various physical parameters can be exploited to enhance contrast in MR images of biological tissues, including T1 and T2 relaxation times, proton density (PD), susceptibility effects, and chemical shift. In T1-weighted images, high free water content distinguishes regions from those with bound water, resulting in positive contrast for damaged tissue. This contrasts with the negative contrast in T2-weighted images. This positive contrast is advantageous for segmentation in low Signal-to-Noise images, common in low-field MRI (Suchanek *et al.*, 2017).

2.3.4 Comparison of the technologies

Table 2.1 compares the different technologies for the quality prediction of fresh fruit and vegetables.

Table 2.1: Comparison of different non-destructive technologies based on their advantages and disadvantages.

Technology	Advantages	Disadvantages
NIRS	(i) Determine chemical and physical parameters without the need for sample preparation. (ii) Provides rapid and real-time information from samples, making it suitable for online sorting and grading in commercial supply chain. (iii) Relatively low cost compared to other technologies.	(i) Lack of model robustness, requiring a new calibration for different fruit species and cultivars, and needing large samples with significant variation for calibration. (ii) Being less accurate for thick rind fruits due to the low penetration depth of NIR radiation. (iii) Relies on spot measurements which can be problematic for heterogeneous fruits with varying qualities within different parts of the fruit. (iv) Chemometric techniques should be applied to extract the information.

Hyperspectral and Multispectral imaging	(i) Provides spatial information.	(i) Online and real-time applications are limited. (ii) Generate vast amounts of data, which require a significant amount of time for image acquisition and complicated analysis.
MRI	(i) Permit repeated analysis at different times to take image slices in any plane through the produce. (ii) Chemical shift can be encoded so that only protons from water, sugar or oil appear in the image and the contrast can be manipulated to display dynamic processes such as flow or self-diffusion. (iii) Allows for the understanding of underlying physiological processes in fruit and vegetables and enables the spatial and temporal study of disorder development. (iv) No sample preparation required.	(i) Requirement for high magnetic fields and sophisticated electronics, which means that the equipment is generally large. (ii) Costly and has a long throughput for scanning and image analysis, which limits its use to research applications. (iii) Not suitable for commercial online sorting and grading, and there are health and safety issues associated with maintaining the high magnetic field.
X-ray CT	(i) Can penetrate fruit tissue without serious surface reflection and has a spatial resolution. (ii) Due to its large field of view, it can scan large samples at a time without sample preparation. (iii) High-resolution three-dimensional images can be constructed, that helps to analyse and visualise microstructure features.	(i) Expensive and complex. (ii) The equipment size is large and there is a potential health hazard due to X-ray leakage. (iii) The data acquisition and image processing time are lengthy and not suitable for commercial online sorting and grading.

	(iv) Allows for the true determination of volumes and densities of desired regions of interest. (v) Enables spatial and temporal study of disorder development and is highly accurate.	
Acoustic	(i) Simple and easy to use. (ii) Quick and precise result. (iii) Cost effective and energy efficient.	(i) High sound intensity may damage the textural quality of products. (ii) Not suitable for online sorting and grading.

2.3.5 Prospects of non-destructive technologies for mango internal disorder prediction / detection

The use of MRI and X-ray CT presents new opportunities for evaluating IDs and understanding their related physiological processes in fruit and vegetables. However, due to high costs, large equipment sizes, health hazards, and slow throughput, they are primarily used for research and not for commercial online sorting and grading. The need for improved real-time detection of quality using these technologies is essential. Similarly, hyper-, and multispectral imaging techniques can generate vast amounts of data, and the relatively complicated image analysis procedures limit their use in online sorting and grading of fruit and vegetables. Acoustic technology has been successfully used for rapid and accurate online detection of IDs in various fruits, but no report has been made on ID detection in mango fruit. Nonetheless, its advantages and accuracy suggest that it could be utilised for online quality prediction of fruit and vegetables. Despite its disadvantages, such as a lack of model robustness and the need for chemometrics to extract information, NIRS has been successfully used to detect the internal and external quality of various fruit and vegetables. Recent studies suggest that NIRS could also be used to detect IDs in mangoes.

2.4 Conclusions

Mango fruit expressed different IDs postharvest. This review highlighted the factors associated with and technologies to predict or detect IDs of mango fruit. Evidence suggests preharvest factors such as nutrient imbalances (particularly N and Ca) are the primary causes

of IDs. Fruit maturity at harvest is also an important factor, with different maturity levels responding differently to postharvest handling practices such as storage temperature, gaseous composition, and VHT, that exacerbate disorders. Fruit maturity in terms of DMC is a reliable objective harvest index that can accurately classify fruit objectively and also correlates with ripe eating. It is important to detect these disorders prior to market supply to ensure consumer satisfaction and to reduce losses. Non-destructive technologies such as NIRS have been identified as useful for rapid, low-cost prediction of internal quality and disorders of mango fruit.

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Chapter 3 Factors contributing to 'B74' mango fruit internal disorders in export supply chains

3.1 Introduction

'Internal disorders', 'internal flesh breakdown', or 'internal breakdown' is a widespread fruit quality problem in many mango cultivars (Arpaia, 1994; Torres *et al.*, 2004; Bally, 2006; Jha *et al.*, 2010; Ma *et al.*, 2018). Various studies reported jelly seed, soft nose, and spongy tissue as the most common forms of IDs. They may have similar underlying causes (Arpaia, 1994; Bally, 2006; Jha *et al.*, 2010). While some specific types of IDs have been given particular names, others are described in generic terms, such as internal flesh breakdown, internal breakdown, or internal disorder, for different types of disorders (Bally, 2006). However, the use of such generic terms in the literature for different types of disorders can create confusion, hindering generalisation of the underlying causes specific to each disorder. To advance understanding of this problem and suggest possible solutions, it is desirable to use accurate descriptors for each specific disorder. Moreover, different studies have suggested various factors or hypotheses that may contribute to making fruit more susceptible to IDs.

Several literature references suggest that factors responsible for IDs include, growing location, nutrient imbalance (particularly calcium (Ca)), fruit maturity at harvest, and postharvest treatments (Bally, 2006). The incidence and severity of VHT-induced IDs are influenced by the growing location (Esguerra *et al.*, 1990). For example, Joyce and Shorter (1994) reported that fruit growing in hotter locations are less sensitive to postharvest heat treatment, as they may have already acclimatized. Several studies suggest that Ca deficiency contributes to internal flesh breakdown in different fruit, including mango (Raymond *et al.*, 1998; Whangchai *et al.*, 2001; Torres *et al.*, 2004; Bally, 2006; Ma *et al.*, 2018). Rainfall close to, or at harvest can decrease fruit tolerance to heat injury (Jacobi *et al.*, 2001). For example, 'Kensington Pride' mango harvested following a prolonged period of heavy rainfall and subjected to VHT (46.5 °C fruit core temperature for 10 mins) exhibited increased skin browning and internal starch layer deposition (Johnson *et al.*, 1992; Jacobi and Wong, 1993; Cooke and Johnson, 1994; Jacobi and Giles, 1997). The severity of disorders increased as the fruit matured. Examples of these disorders include watery pulp breakdown (Bally, 2006), stem end cavity (Hofman and Whiley, 2010), and jelly seed in mango (Yahia, 1998; Yahia, 2005; Hofman and Whiley, 2010). Fruit response to VHT is also affected by the maturity (Esguerra

and Lizada, 1987). Less mature fruit are more prone to VHT-induced internal breakdown than are mature fruit (Esguerra *et al.*, 1990; Jacobi *et al.*, 2001).

Specific factors (pre-harvest or post-harvest) responsible for making fruit sensitive or tolerant to IDs in 'B74' mango fruit haven't been determined. Hence, understanding environment and management interactions before and after harvest is important. Towards addressing this lack of insight, two approaches were followed in this study: i) survey (growers and postharvest fruit handlers) and ii) monitoring of IDs in different supply chains from different growing regions and within orchard blocks. These approaches were to identify when, where, and which IDs have occurred, and to collect information about environmental and cultural practices associated with occurrence or absence of the disorders.

3.2 Methodology

3.2.1 Survey

3.2.1.1 Grower survey

A structured questionnaire was developed (Appendix 6) with ethics approval from UQ (Appendix 8). Only two growers export 'B74' fruit, one from Northern Territory (NT) and the other from North Queensland (NQ). All other 'B74' growers supply fruit only to the domestic market, which does not require VHT. Therefore, information was collected only from these two farms. It related to the environment, edaphic, cultural practices, and harvest. Meteorological information for each farm was obtained from the nearest station of the Australian Government Bureau of Meteorology (BOM; http://www.bom.gov.au/climate/averages/tables/ca_qld_names.shtml). Data from Tindal RAAF (Station number: 014932) and Walkmin Research Station (Station number: 031108) were used for NT and NQ farms, respectively.

3.2.1.2 Postharvest fruit handler survey

The production and marketing rights for the 'B74' mango fruit is vested with Perfection Fresh (PF), Australia, and they are responsible for the postharvest handling of the fruit. Therefore, the survey was with their personnel involved in fruit handling and was conducted to collect information about the postharvest handling operations associated with occurrence or absence of IDs. Internal reports and anecdotal information were also collected. During the survey, it was found that IDs were also an issue in the domestic supply chains in

the previous year. Therefore, postharvest handling information relating to the domestic supply chains was also collected. The structured questionnaire was prepared (Appendix 7) and approved by The University of Queensland (UQ) ethics committee (Appendix 8).

3.2.2 Supply chain monitoring

Supply chain monitoring was conducted for three harvest seasons (2020 - 2023). Fruit was sourced from two major mango production regions of Australia, the NT and NQ. The aim of monitoring was to identify the different types of IDs and factors that contribute to 'B74' IDs along the export supply chains for fruit from different origins. Thus covering, region, farm, block, and supply chain overall.

3.2.2.1 Fruit source

Green-mature 'B74' mango fruit were harvested from commercial farms in the NT and NQ, Australia. They were grown, harvested, de-sapped, fungicide treated, graded, and packed according to commercial practice. The fruit were transported from farms to a commercial VHT facility at Rocklea, Brisbane. The distances involved were *ca*. 3424 and *ca.* 1720 km for NT and NQ fruit, respectively. Transit time from packing shed to VHT and temperature were monitored only in the 2021/22 and 2022/23 harvest seasons. Nine, 27, and seven supply chains were monitored in 2020/21, 2021/22, and 2022/23 harvest seasons, respectively. In this context, 'supply chain' refers to consignments individually sampled upon arrival at the VHT facility and numerically labelled (e.g., supply chain 1, 2, 3).

3.2.2.2 Sampling

Random sampling of fruit for allocation to treatments was performed at the VHT facility. Sample size was calculated by $n = \frac{z^2 \times p(1-p)}{\varepsilon^2}$ (Cochran, 1977); where, n = sample size, z = 1.96 for 95% confidence level, p = population proportion, assumed it to be 50% when not known, and Ɛ = margin of error. The calculated sample size was 97 for a 10% margin of error. For uniform representation in each treatment and replicates, 102 fruit per supply chain were randomly sampled. Of these, 51 were allocated as untreated controls and the others for VHT. Each treatment was replicated three times, with 17 fruit per replication.

3.2.2.3 Disinfestation vapour heat treatment (VHT)

VHT was done in the commercial VHT facility of PF Rocklea, Brisbane. Fruit were tipped into bins and placed in the commercial VHT chamber. A single pulp-temperature probe

was inserted into the centre of a random fruit in the middle of each bin. VHT was applied to fruit in the bin as per the commercial protocol, viz., fruit core temperature maintained at 47 °C for 15 mins with >90% relative humidity (RH) (Hofman, 2011). The temperature of the fruit was gradually increased (Appendix 45) from ~18 °C to 47 °C and RH was ~88% to 93% within the *ca.* 4 h of treatment. Thereafter, fruit were maintained at that temperature and RH for 15 mins to complete the disinfestation process. Fruit were removed from the treatment chamber immediately after treatment.

3.2.2.4 Fruit maturity, firmness, and shelf-life

Immediately after VHT, all fruit were transported in an air-conditioned vehicle to the postharvest laboratory at The University of Queensland, Gatton (~72 km; ~1 h). Individual fruit maturity in terms of dry matter content (represented as a percentage of dry weight to fresh weight; DMC) was assessed by portable near infra-red (NIR) (F-750 Produce Quality Meter, CID Bio-Science, Inc., Washington, USA) device (Felix Instruments, 2018) immediately on arrival. In 2020/21, individual fruit DMC was not assessed by NIR in supply chains 1, 2, 4, and 5. In these supply chains, 20 fruit were randomly sampled, and their DMC assessed by the oven-dry method (Padda *et al.*, 2011). Thereafter, all fruit were held at ~20 °C and ~90% RH until reaching the eating soft stage. This stage was assessed by hand firmness ratings of 0 - 4 (Hofman *et al.*, 2010). The time taken for fruit to reach firmness stage 4 (eating soft) was considered its shelf-life.

3.2.2.5 Internal disorders

At the eating soft stage, individual fruit were sliced longitudinally on both sides of the seed and assessed visually for incidence and severity of the IDs flesh cavity with white patches (FCWP) and flesh browning (FB). FCWP and FB severity for individual fruit was rated on a scale 0 - 3 (Appendix 9).

3.2.2.6 Experiment design and analysis

In this monitoring trial, each supply chain was considered an independent experiment with two treatments i.e., non-VHT and VHT. An unpaired, *t*-test was utilized at $P<0.05$ to compare the differences in the incidence and severity of IDs, and shelf-life between the two treatments. Additionally, Pearson's correlations were made between the individual fruit Nir-DMC and the severity of FCWP and FB, as well as between individual fruit shelf-life and FB.

All supply chain data for each year was pooled. Analysis of variance between supply chains in incidence and severity of IDs was performed, and means were separated by Tukey's HSD at $P<0.05$. In each harvest season, fruit were sourced from the two different growing regions (NT and NQ). Each region has different growing orchard blocks, and each block has a different number of supply chains. Therefore, to examine effects of these factors on incidence and severity of IDs, data from all supply chains for each year were pooled. The variation in IDs incidence and severity across the season, region, and blocks were evaluated using the linear mixed model (LMM) in the R package 'lme4'.

Time from packing shed to VHT facility (h), ambient transit temperature (°C), postharvest time and temperature unit sums (TTUs) and individual fruit Nir-DMC were recorded for each supply chain in the 2021/22 harvest season. To examine relationships for the above-mentioned parameters with incidence and severity of IDs, correlations between these parameters for FCWP and FB was conducted.

3.3 Results

3.3.1 Grower survey

This section provides a concise description of information collected during the grower survey that relates to cultural practices, harvest and postharvest practices, edaphic conditions, and meteorological factors.

3.3.1.1 Cultural practices

Annual light pruning was conducted post-fruit harvest, consisting of the elimination of tops, lateral shoots, and internal branches. Towards optimal flowering, irrigation was withheld prior to flowering and then initiated at 1500 L per tree per week from micro-sprinklers for 3 h daily once 50% of the flowers had opened. Irrigation was later reduced, but not fully discontinued, 4 - 5 d prior to strip-pick harvest. The nutrient management strategies employed by both farms were similar but differed in sources utilised (Appendix 11). Same nutrient management plans were implemented for different blocks, despite variations in soil test parameters between the blocks (Appendix 12).

3.3.1.2 Harvest and postharvest practices

The appropriate time for harvest was determined considering multiple criteria, including DMC (NT grower target 16%; NQ grower target 14%), flesh colour (7), and

accumulated heat sums (1680 and 1480 h from ‘asparagus’ and ‘Christmas tree’ stages, respectively). Harvest was conducted throughout the day and fruit reached packing sheds within 5 h of harvest. They were then stored overnight under ambient conditions prior to being packed the following day. The fruit were then subject to forced air cooling at 16 °C before being sent to the VHT facility in Rocklea, Brisbane. On occasion, the fruit were held at that same temperature (16 °C) for up to 24 h. A refrigerated truck, maintained at 16 °C, was utilised to transport the fruit from the packing shed to the VHT facility.

3.3.1.3 Edaphic and meteorological parameters

Obtaining edaphic information from the growers was challenging, they were hesitant to share such information. However, information from the growers regarding soil test results for various farms and blocks are presented in Appendix 12. The soil of the NT farm was alkaline, while of the NQ farm was neutral. All nutrients, except zinc, were judged below optimal in the soil test reports (Appendix 12).

Flowering time for the NT farm was June - July, with harvest in October - November. The NQ farm flowering time was August - September and harvest time November - December. Daily maximum and minimum temperature and daily rainfall from flowering to harvest for both regions are presented in Appendix 10. The graphs therein show that daily maximum and minimum temperatures in the NT region were higher than those in the NQ region. The maximum daily temperature during the flowering period in NT was 30 °C, whereas it was 25 °C in NQ. And during fruit harvest it was 40 °C and 33 °C in NT and NQ, respectively. The NT region was relatively drier during the fruit harvest period in all four fruiting seasons compared to NQ (Appendix 10).

3.3.2 Postharvest fruit handler survey

Upon fruit arrival at the VHT facility, fruit are usually held at 13 - 16 °C for 4 - 28 h and VHT practised as in Section 3.2.2.3. Transit time from packing shed to VHT was 3 - 11 d and 2 - 7 d in the 2018/19 and 2019/20 harvest seasons, respectively. In 2019/20 specifically, transit time for NT fruit were on average 5.5 d (ranging from 5 - 7 d), while for the NQ fruit it was 3.5 d (ranging from 2 - 4 d). Proportion of grade-out due to obvious skin injury after VHT was higher (30.9% and 12.5% in NT and NQ fruit, respectively) in the 2018/19 season compared to the 2019/20 (22.2% and 10.7% in NT and NQ fruit, respectively). VHT in the fruit more than 5 d from packing to VHT was found to have more than 10% grade out

proportion (Appendix 22). Cold chain was not in place in the 2018/19 season however, it was improved in 2019/20.

3.3.2.1 History of internal disorders

In 2018/19 jelly seed and internal browning significantly affected the fruit in the domestic supply chain. A trace-back study conducted by PF during this season revealed that fruit were exposed to higher pre-harvest temperatures, were over-mature, and experienced higher packing shed temperatures ranging between 40 - 42 °C. Fruit were 15 - 25+ d after packing during assessment. Occurrence of internal browning was observed only in fruit handled by one ripening centre. Fruit exported to New Zealand with gamma irradiation also had FB. The grower survey also revealed that fruit in the library trays did not express IDs, whereas fruit transported to the market did.

In 2018/19 fruit were air and sea freighted for export. FCWP was only disorder observed in air freighted fruit. The time between VHT to the fruit arrival in the export market ranged from 2 - 7 d. However, in sea freighted (CA; 3% O_2 and 5% CO_2; container at 11 °C) fruit had both FCWP and FB. Tracing back the postharvest handling operation in those sea freighted consignments revealed time from packing to VHT was 8 - 11 d and they were 31 - 36 d from packing to they arrived in the export market. Fruit with more transit time (34 - 36 d) had relatively higher FB (60 - 85%) and whereas fruit with less transit time (31 - 33 d) had relatively less FB (20 - 35%).

In 2019/20 there was no IDs issues in domestic supply chains and air frighted export fruit. In sea freighted fruit there were signs of compromised fruit quality caused by IDs, but overall quality was acceptable upon arrival. Transit time from packing to VHT was 4 - 7 d compared to 8 - 11 d in 2018/19. Time from harvest to export market was reduced almost by 10 d in 2019/20 compared to 2018/19.

3.3.3 Monitoring trial

3.3.3.1 Fruit weight

The results of the monitoring trial revealed a range in fruit weight across different supply chains and individual harvests. For instance, in the monitoring trial of 2020/21, in supply chain 1, the fruit weight ranged from 405 - 585 g, with an average weight of 481 g. In supply chain 4, weight ranged from 411 - 594 g with an average of 489 g. In supply chains, 2,

3, and 5 to 9, relatively less variation in fruit weight was observed. Least variation in fruit weight was observed in supply chains 2 and 7, where fruit weight ranged from 456 - 504 g with an average of 477 g and 433 - 482 g with an average of 459 g, respectively (Figure 3.1, right). Fruit weight from different supply chains and within each harvest in the 2021/22 and 2022/23 monitoring trial also varied considerably (Appendix 13).

3.3.3.2 Fruit maturity (DMC)

The monitoring trial in 2020/21 revealed the DMC distribution of fruit from different supply chains and within each harvest varied. More than 50% of fruit in supply chains 1, 4, 7, 8, and 9 had DMC ≤15%. In supply chains 2, 3, 5, and 6, fruit DMC was mostly >15%. DMC also varied widely among individual harvests. For example, in supply chain 3, fruit DMC ranged from 14.4% - 19.9%. Similar variation was observed in fruit from remaining supply chains (Figure 3.1, left). Fruit DMC from different supply chains and within each harvest in the 2021/22 and 2022/23 monitoring trial also varied considerably (Appendix 14).

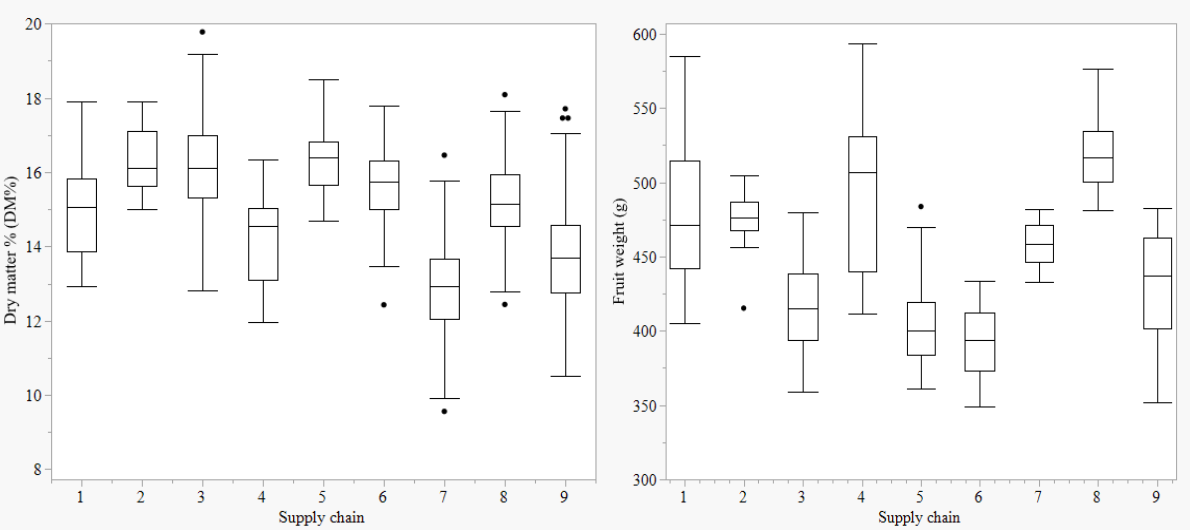


Figure 3.1: 'B74' mango fruit dry matter content % (DMC) (left) and fruit weight (right) distribution in supply chains covering two regions of Australia i.e., Northern Territory (NT) and North Queensland (NQ) in the 2020/21 harvest seasons. Dots represent outliers. Ends of lower and upper whiskers represent minimum and maximum data values, respectively. Lower whiskers represent 25% data set. Upper whisker represents the 25% data set. Boxes represent the intermediate 50% data set. Transverse line within the boxes is the median. Diagram was constructed in JMP Pro 16.0.0.

3.3.3.3 Internal disorders

During the three-year monitoring trial, it was observed that 'B74' mango fruit in export supply chains exhibited three IDs: FC, FCWP, and FB (Figure 3.2). While FC was not severe and did not affect the marketability of the fruit, FCWP and FB were severe and deemed to be of economic importance. Interestingly, the disorders reported during the survey, such as jelly seed and stem end cavity, were not observed in the monitoring trial. This discrepancy may be attributed to inconsistent naming of the same disorder or different names given to similar disorders. Therefore, the major disorders observed during the study were defined and shared herein in order to avoid potential confusion with the other disorders and generic names, such as 'internal disorders', 'internal breakdown', and 'flesh breakdown'. FCWP is characterised by the presence of cavities with white, starchy, and tough lesions in the mesocarp near the seed (Figure 3.2; middle). FB is characterised by the presence of diffuse brown discolouration of the flesh, usually near the seed (Figure 3.2; right).



Figure 3.2: Internal disorders that were observed during supply chain monitoring; flesh cavity (FC) (left); flesh cavity with white patches (FCWP) (middle), and flesh browning (FB) (right).

3.3.3.3.1 Flesh cavity with white patches

The results from the three-year (2020/21 - 2022/23) monitoring trials revealed that FCWP was observed exclusively in VHT fruit (Appendix 21). Considerable variation was observed in FCWP incidence and severity among growing regions, harvest seasons, different growing blocks, and different supply chains. For example, NT had significantly *($P<0.05$)* lower FCWP than NQ. FCWP in 2022/23 was significantly *($P<0.05$)* lower than 2020/21 and 2021/22. Orchard blocks M38-2, M38-3 and M56-1had significantly *($P<0.05$)* lower FCWP than M36-1, M36-2, M36-3, and M36-4 (Table 3.1).

In 2020/21, a significant variation *(P<0.05)* in FCWP incidence was observed among the different supply chains (Figure 3.3). Specifically, supply chain 6 had a significantly higher FCWP incidence (74.1%) and was similar to supply chain 9 (66.2%). In contrast, supply chain 3 had a lower *(P<0.05)* FCWP incidence (1.96%) and was similar with the supply chains 1, 5, and 8. Supply chains 1, 2, and 3 were from the same harvest block (M38-2) but harvested at different times, and supply chain 3 had a lower *(P<0.05)* FCWP incidence than supply chains 1 and 2, and FCWP in the latter two supply chains were similar. Supply chains 6 and 7 were from the same harvest block (M36-3) but were harvested at different times and had different *(P<0.05)* FCWP incidence (Figure 3.3). Similar variation in FCWP incidence among the different supply chains originated from the same orchard blocks was observed in the 2021/22 and 2022/23 monitoring trials (Appendices 15 and 16).

Table 3.1: Effect of treatment (+/-vapour heat treatment (VHT)), region (Northern Territory (NT) and North Queensland (NQ)), harvest season (2020/21, 2021/22 and 2022/23) and harvest orchard blocks (M36-1, M36-2, M36-3, M36-4, M38-2, M38-3, and M56-1) on flesh cavity with white patches (FCWP) incidence of 'B74' mango fruit in 3-year monitoring trials. In the first, second, and third year, nine, 27, and seven supply chains were respectively monitored with each supply chain involved sampling 102 fruit.

Variable	Incidence %	Standard error (SE) %	Adjusted SE %	Z value	*P* value
Intercept	8.1	5.2	5.2	1.6	0.11
Treatment VHT	39.8	2.5	2.5	15.8	0.00***
Region NT	-18.7	2.6	2.6	7.3	0.00***
Harvest season 2021/22	4.8	3.5	3.5	1.4	0.16
Harvest season 2022/23	-9.0	4.6	4.6	1.7	0.04*
Block M36-2	0.7	9.8	9.9	0.1	0.94
Block M36-3	-6.1	9.0	9.1	0.7	0.50
Block M36-4	-14.8	11.3	11.4	1.3	0.19
Block M38-2	-29.3	10.2	10.3	2.8	0.00**
Block M38-3	-25.0	9.3	9.3	2.7	0.00**
Block M56-1	-22.1	9.4	9.4	2.3	0.02*

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

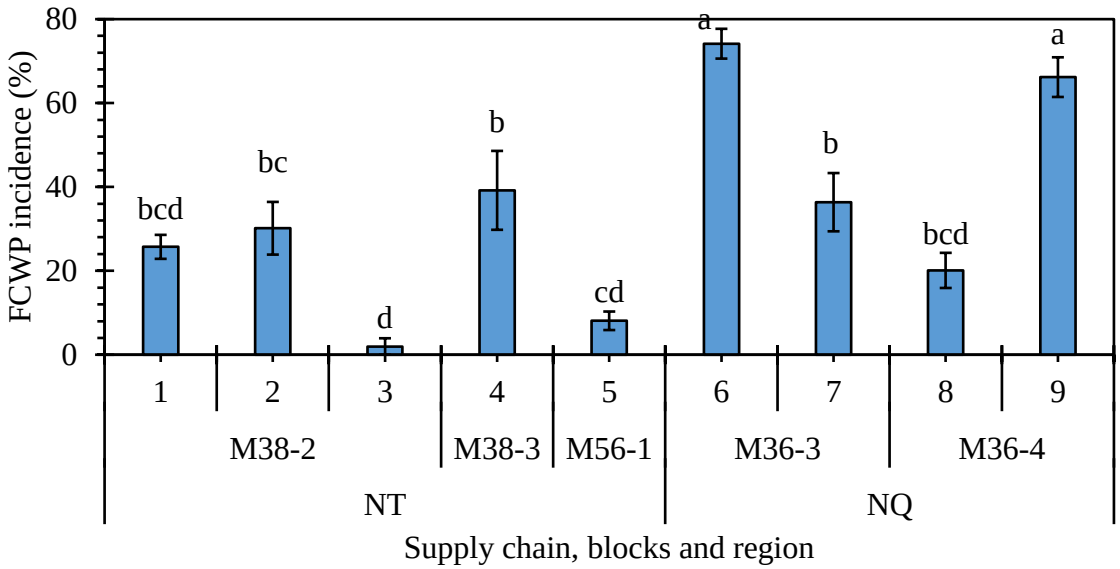


Figure 3.3: Incidence of flesh cavity with white patches (FCWP) in nine separate case study supply chains during the 2020/21 harvest season with vapour heat treatment (VHT). The data were collected from two regions in Australia, Northern Territory (NT) and North Queensland (NQ) with different harvest blocks from each region. Levels not represented by same letter are significantly different at *P<0.05*. Vertical bars represent ± standard error (SE) of means.

3.3.3.3.2 Incidence and severity of FB

The monitoring trials conducted over three seasons (2020/21 - 2022/23) revealed that FB was in both VHT and non-VHT fruit and had significant effect. VHT fruit had lower *(P<0.05)* FB incidence than non-VHT fruit (Table 3.2). Considerable variation in FB incidence existed between different orchard blocks, harvest seasons, and supply chains. For example, FB in the 2022/23 harvest seasons had higher *(P<0.05)* than the 2020/21 and 2021/22 harvest season. Similarly, harvest block also different *(P<0.05)* in FB incidence (Table 3.2). In the 2020/21 season, a significant variation *(P<0.05)* in FB incidence was observed among the different supply chains (Figure 3.4). All the supply chains originated from NT, except supply chains 1 and 5, showed a significantly higher FB incidence than those from the NQ region. FB incidence in supply chains 1 and 5 were similar to all the supply chains originating from NQ (Figure 3.4). Variation in FB incidence among the different supply chains originated from the same orchard blocks was observed in the 2021/22 and 2022/23 monitoring trials (Appendices 17 and 18).

Table 3.2: Effect of treatment (+/-vapour heat treatment (VHT)), region (Northern Territory (NT) and North Queensland (NQ)), harvest season (2020/21, 2021/22 and 2022/23) and harvest orchard blocks (M36-1, M36-2, M36-3, M36-4, M38-2, M38-3, and M56-1) on flesh browning (FB) incidence of 'B74' mango fruit in 3-year monitoring trials. In the first, second, and third year, nine, 27, and seven supply chains were respectively monitored with each supply chain involved sampling 102 fruit.

Variable	Incidence %	Standard error (SE) %	Adjusted SE %	Z value	*P* value
Intercept	64.8	11.4	11.5	5.6	0.00***
Treatment VHT	-13.5	2.7	2.7	4.9	0.00***
Region NT	-5.1	3.1	3.1	1.6	0.11
Harvest season 2021/22	8.3	4.4	4.4	1.9	0.06
Harvest season 2022/23	16.6	5.6	5.6	3.0	0.00**
Block M36-2	-32.8	10.8	10.8	3.0	0.00**
Block M36-3	-38.3	10.0	10.0	3.8	0.00***
Block M36-4	-55.5	13.0	13.1	4.3	0.00***
Block M38-2	-41.1	11.7	11.8	3.5	0.00***
Block M38-3	-56.8	10.3	10.3	5.5	0.00***
Block M56-1	-26.2	10.4	10.4	2.5	0.01*

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

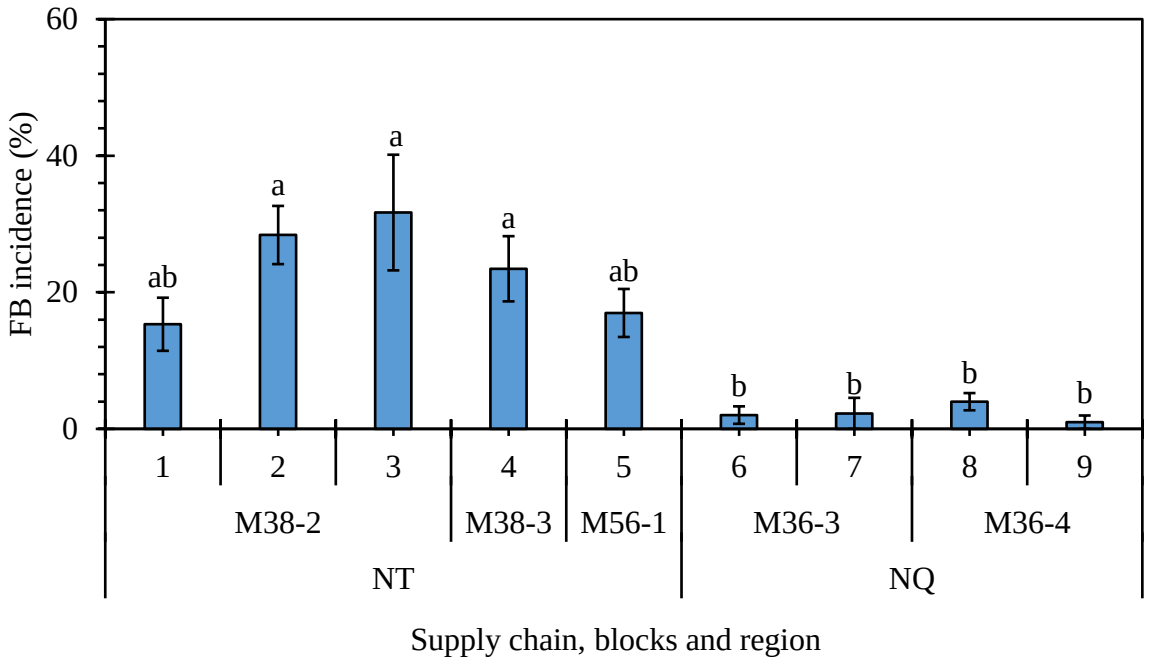


Figure 3.4: Flesh browning (FB) incidence in nine separate case study supply chains during 2020/21 harvest season. The data were collected from two regions in Australia, Northern Territory (NT) and North Queensland (NQ) with different harvest blocks from each region. Levels not represented by same letter are significantly different at *P<0.05*. Vertical bars represent ± standard error (SE) of means.

3.3.3.4 Shelf-life

The effect of VHT on the shelf-life of 'B74' mango fruit was investigated in different case study supply chains in three seasons (2020/21, 2021/22, and 2022/23). The results of the 2020/21 case study supply chain revealed no significant (*P*>0.05) difference in the effect of VHT across all supply chains (Table 3.3). However, and except for supply chains 2 and 6, non-VHT fruit exhibited relatively longer shelf-life than VHT fruit (Table 3.3). This tendency was also observed in the 2022/23 case study supply chains, except for supply chain 4, there was no difference *(P>0.05)* in shelf-life between two treatments (Appendix 19). However, in 2021/22 supply chains 1, 2, 3, 4, 5, 6, 7, 11, 17, 20, and 23, non-VHT fruit had a significantly *(P<0.05)* longer shelf-life than VHT fruit (Appendix 20).

Table 3.3: Effect of +/- vapour heat treatment (VHT) on shelf-life (mean ± SE) of 'B74' mango in nine case study supply chains during the 2020/21 harvest season. Shelf-life was calculated from the day of VHT to the fruit reaching hand firmness rating scale of 4. No data in supply chain seven as only VHT fruit was available during monitoring. In each supply chain, 102 fruit were evenly distributed between two treatments, with 51 +VHT and 51 -VHT).

Supply chain	Shelf-life (d)		
	+VHT	-VHT	Significance
1	12.3 ± 0.7	15.6 ± 1.3	NS
2	14.5 ± 0.4	13.9 ± 0.9	NS
3	13.7 ± 0.3	16.3 ± 1.2	NS
4	16.2 ± 0.6	18.5 ± 1.1	NS
5	16.7 ± 0.7	17.5 ± 0.9	NS
6	14.1 ± 0.2	13.5 ± 0.6	NS
7	-	-	-
8	11.0 ± 0.7	12.8 ± 0.6	NS
9	12.5 ± 0.1	12.9 ± 0.3	NS

NS: non-significant.

3.3.3.5 Relationship between DMC, FCWP and FB; and shelf-life and FB

The monitoring trial conducted in the 2020/21 season revealed that fruit from supply chains with higher average DMC generally exhibited a comparatively lower incidence of FCWP and vice versa (Figure 3.5). For example, supply chains 3 and 5 had lower incidence of FCWP and higher fruit DMC. Conversely, supply chains 4, 7, and 9 had higher FCWP and relatively lower average DMC (<14.2%). Supply chains 1 and 2 were from the same block but harvested at different times but earlier than chain 3. Fruit harvested earlier had a relatively lower DMC that coincided with their higher FCWP incidence compared to late harvested fruit with higher DMC (Figure 3.5).

During 2021/22, majority of the supply chains displayed negative correlations between FCWP severity and fruit DMC especially in supply chains 17, 18, 21, 22, 24, 25, and 27, there was a significant negative correlation (Table 3.4). These results suggest that fruit maturity may be one of the important factors that make fruit sensitive to VHT-induced FCWP.

In 2020/21, fruit from supply chains having higher average DMC generally exhibited relatively higher incidence of FB (Figure 3.6). For example, in 2020/21, supply chains 2, 3, and 5 had higher incidence of FB corresponding to relatively higher average fruit DMC. Reciprocally, fruit from supply chains having lower average DMC (<15%) generally exhibited relatively lower FB incidence. Supply chains 1, 2, and 3 were from the same block, but harvested at different times, with chain 1 earlier followed by 2, and 3 being harvested later. Earlier harvested fruit had relatively lower DMC that coincided with their lower FB incidence compared to late harvested fruit with higher DMC (Figure 3.6). In the 2021/22 season, FB severity was positively correlated with individual fruit DMC in all other supply chains (Table 3.4).

In the course of three harvest seasons, there was a correlation between the fruit shelf-life and FB severity across majority of monitored supply chains. During the 2021/22 harvest season, positive correlation was observed between FB severity and shelf-life in all monitored supply chains (Table 3.4).

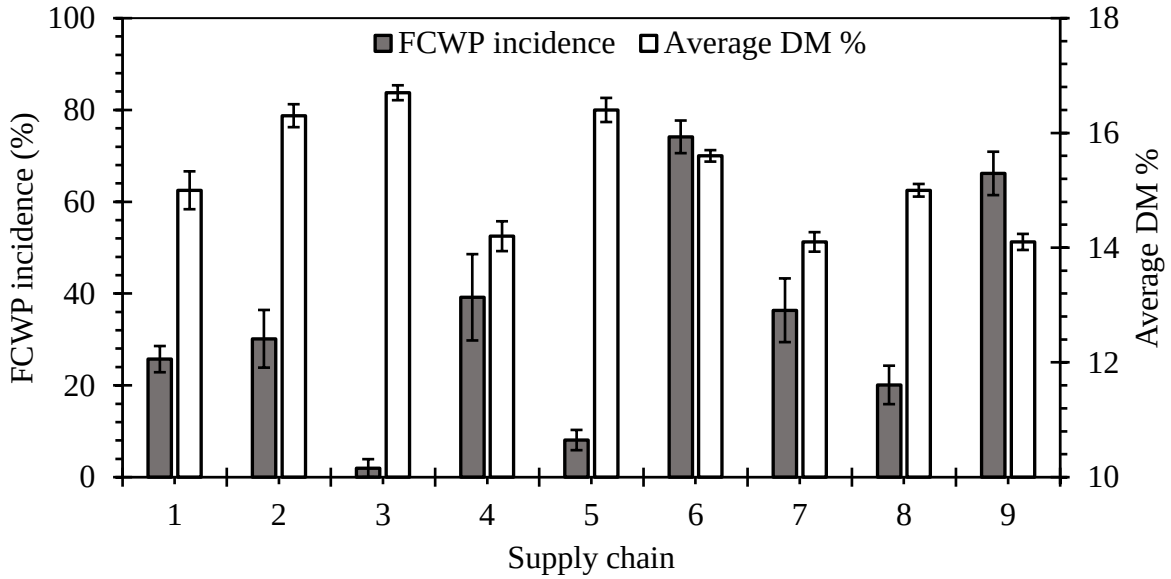


Figure 3.5: Average dry matter content (DMC) (n = 102) and flesh cavity with white patches (FCWP) incidence (%) in nine different case study supply chains for 'B74' mango fruit subjected to vapour heat treatment (VHT). Fruit were collected from two different regions in Australia i.e., Northern Territory (NT), North Queensland (NQ). FCWP incidence % presented is the sum of incidences for three severity classes; viz., slight, moderate, and severe. Vertical bars are the ± standard error of mean.

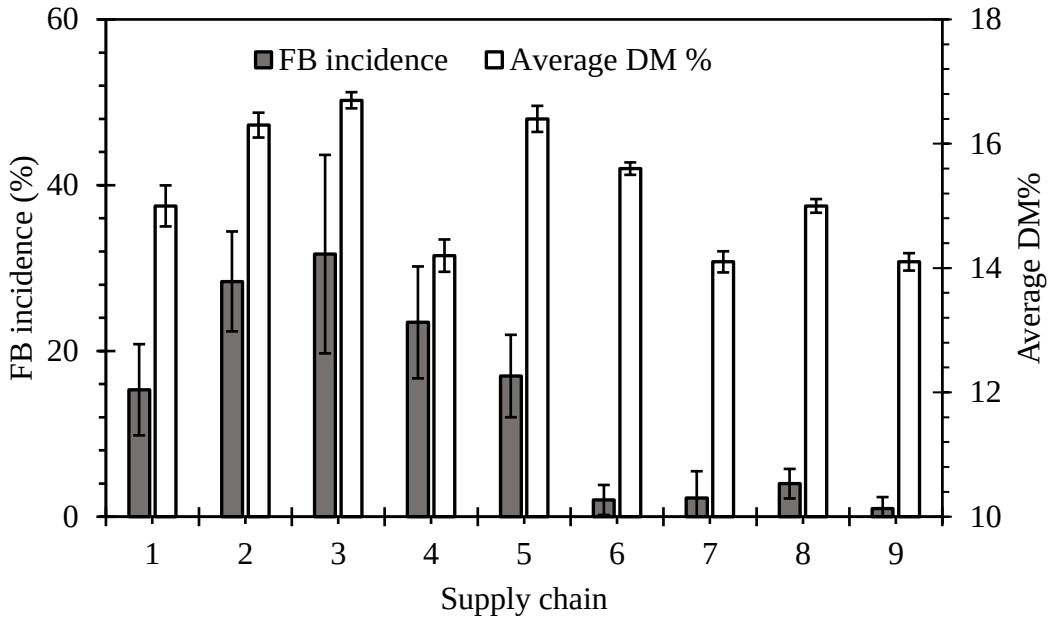


Figure 3.6: Average dry matter content (DMC) (n =102) and Flesh Browning (FB) incidence (%) in nine different case study supply chains for 'B74' mango fruit. Fruit were collected from two different regions in Australia i.e., Northern Territory (NT), North Queensland (NQ). FB incidence % presented is the sum of incidences for three severity classes; viz., slight, moderate, and severe. Vertical bars are the ± standard error of mean.

Table 3.4: Correlation between individual fruit DMC from each supply chain with the flesh cavity with white patches (FCWP) and flesh browning (FB) severity in 27 different case study supply chains monitoring during 2021/22 harvest seasons. FB severity for individual fruit was rated on a scale 0 - 3.

	Supply chain	FCWP	FB		Supply chain	FB
DMC	1	-0.29	0.35**	Shelf-life	1	0.21
	2	0.17	0.24*		2	0.26*
	3	-0.16	0.02		3	0.21*
	4	0.2	0.26*		4	0.12
	5	0.21	0.29**		5	0.15
	6	-0.04	0.00		6	0.00
	7	0.49***	0.00		7	0.18
	8	0.05	0.00		8	0.00
	9	0.18	0.18		9	0.08
	10	-0.12	0.00		10	0.27*

	11	0.00	0.07	11	0.41***
	12	0.08	-0.09	12	0.09
	13	-0.12	0.23	13	0.33***
	14	-0.04	0.00	14	0.00
	15	-0.21	0.31**	15	0.16
	16	0.00	0.16	16	-
	17	-0.44**	0.36***	17	0.27***
	18	-0.38**	0.32**	18	0.27***
	19	-0.03	-0.02	19	0.43***
	20	-0.11	0.45***	20	0.40***
	21	-0.44**	0.34***	21	0.16
	22	-0.34*	0.23*	22	0.33***
	23	-0.24	0.33*	23	0.33***
	24	-0.28*	-0.01	24	0.12
	25	-0.40*	0.32***	25	0.17
	26	-0.11	0.05	26	0.21*
	27	-0.43**	0.20*	27	0.48***

Significance levels *: 0.05, **: 0.01; ***: 0.001

3.3.3.6 Correlation between DMC, postharvest TTUs, and time from packing shed to VHT with FCWP and FB

Correlation between different parameters i.e., DMC, postharvest TTUs, time from packing shed to VHT, that was recorded during the monitoring trial in 2021/22 with the incidence of FCWP and FB is presented in the Table 3.5. No significant correlation was observed between the FCWP incidence and time from packing shed to VHT, postharvest TTUs before VHT and the fruit DMC. In contrast, FB incidence showed significant positive correlation with the time from packing shed to VHT, postharvest TTUs, and DMC (Table 3.5).

Table 3.5: Correlation between time from packing to VHT facility, postharvest time and temperature unit sums (TTUs) from packing to VHT and the individual fruit DMC with the flesh cavity with white patches (FCWP) and flesh browning (FB) incidence in twenty-seven different case study supply chains monitored during the 2021/22 harvest season. Postharvest TTUs is the product of average daily temperature with time.

		Correlation (r)	Probability value
Time (h)	FCWP incidence	0.05	0.79
	FB incidence	0.49**	0.00
Postharvest TTUs	FCWP incidence	-0.04	0.83
	FB incidence	0.39*	0.05
DMC	FCWP incidence	0.15	0.45
	FB incidence	0.61***	0.00

Significant codes: *: 0.05, **: 0.01; ***: 0.001

3.4 Discussion

3.4.1 Flesh cavity with white patches

The study outcomes demonstrate that occurrence of FCWP can be attributed to VHT, since the disorder was found exclusively in fruit subject to VHT (Appendix 21). However, not all VHT fruit displayed FCWP and its absence in some supply chains, even in VHT fruit (Appendices 15 and 16), indicate that its incidence is influenced, if not determined, by pre-VHT factors. Fruit intrinsic quality established pre-VHT is largely determined by pre- and post-harvest factors (Chapter 2; Figure 2.5).

Early harvested fruit with relatively low DMC had higher FCWP incidence than did late-harvested fruit with higher DMC (Figure 3.5). The incidence of FCWP showed negative relationship with the DMC (Table 3.4). Highest correlation being r = - 0.44, $P<0.001$ (Table 3.4) suggested that maturity contributed partly for FCWP and factors beyond maturity may also be involved in making fruit sensitivity to VHT.

Based on fruit sampling from these supply chains, it is evidently important to conduct controlled experiments to identify, compare, and contrast predisposing additional to maturity that are associated with FCWP in ‘B74’ supply chains.

A significant difference was evident for incidence of FCWP between the fruit from NT and NQ (Table 3.1). Across the three seasons monitored, fruit from NT had lower incidence of FCWP than did fruit from NQ (Table 3.1). Joyce and Shorter (1994) reported that growing locations influence sensitivity to heat treatment, with fruit growing in hotter locations showing less sensitivity to heat as they may have been already acclimatized. However, the present study did not definitively establish effects of specific regional or growing conditions on FCWP incidence. Notably, fruit from the same harvest block or growing conditions showed significantly different FCWP incidence. For example, in the 2020/21 monitoring trial, supply chains 1, 2, and 3, originating from the same block (M38-2), exhibited varied FCWP incidence, as did supply chains 6 and 7 in addition to 8 and 9, originating from the same blocks, namely M36-3 and M36-4, respectively (Figure 3.3). Similarly, in the 2021/22 monitoring trial, despite supply chains 1 to 5, 6 to 13, and 15 to 25 originating from the same blocks, significant variations in FCWP incidence were observed among supply chains (Appendix 15).

The disparity in FCWP incidence in two regions may be associated with differences in the transit time from harvest to VHT. Fruit from the NT had a comparatively longer transit time (5 - 7 d) from harvest to VHT, compared to the shorter (3 - 4 d) for fruit from NQ. Holmes (2019) found that time from packing to VHT was the major contributing factor to damage caused by VHT. The role of the physiological stage of fruit in making fruit more tolerant to disinfestation heat treatment is explained by Jacobi *et al.* (1995).

The present survey also determined that >5 d after packing was positively associated with higher skin scalding of fruit during VHT (Appendix 22). Therefore, it is necessary to identify the optimum transit time from harvest to VHT to minimise both FCWP and skin scalding.

Furthermore, VHT applied to fruit of different physiological stage within a single batch may precipitate differential effects. If ripe fruit were used as temperature probe fruit during VHT, then treatment time was extended by as much as 30 mins (Holmes 2019). The temperature lag in ripe fruit may result from compositional changes and density variations. Controlled experiments with fruit of same maturity and different physiological stage of fruit at the time of VHT could help elucidate this phenomenon.

Fruit size in single VHT batches varied (Figure 3.1, right). Sivakumar *et al.* (2011) reported that smaller fruit were more susceptible to heat damage than larger fruit, which was

further supported by Jacobi and Giles (1997) working on ‘Kensington Pride’ mango. Smaller fruit heated up faster and are exposed to the treatment temperature for a longer duration, making them more susceptible to heat damage. Using smaller fruit as a temperature probe fruit during treatment in the heterogenous fruit weight batch can lead to insufficient treatment of the larger fruit, and larger fruit as probe fruit led to the exposing of smaller fruit to treatment temperature longer than required. Therefore, it is recommended to use fruit of similar size during treatment to reduce the negative effects of VHT and ensure proper treatment.

3.4.2 Flesh browning

FB was observed both in VHT and non-VHT fruit and the effect was non-significant in 2020/21 (Appendix 23). However, in 2021/22 certain supply chains (10 of the 27), non-VHT fruit exhibited a significantly higher incidence of FB than VHT fruit (Appendix 24). Additionally, the survey found evidence of FB in domestic supply chains during the 2018/19 season that did not involve VHT (Section 3.3.2.1). Likewise, in fruit exported to NZ that underwent gamma irradiation (Section 3.3.2.1).

This study did not definitively affirm the hypothesis that VHT is the primary cause of FB disorder in ‘B74’ export supply chains. However, contrasting results were obtained across supply chains. FB was observed in fruit samples regardless of whether they had undergone VHT. Notably, non-VHT fruit had higher FB than did VHT treated fruit (Table 3.2). Similarly, Brecht *et al.* (2019) reported no role of quarantine HWT in the development of corte negro, a mango fruit disorder similar to FB.

Nevertheless, Esguerra *et al.* (1990), Esguerrra and Lizada (1990), and Mitcham and McDonald (1993) reported that VHT did damage mango fruit due to internal O_2 deficiency leading to undesirable fermentation products and internal breakdown similar to FB.

Fruit with a higher DMC exhibited a greater susceptibility to FB compared to those with a lower DMC. Likewise, early harvested fruit with relatively lower DMC exhibited lower FB incidence than did late harvested fruit with higher DMC (Figure 3.6). Results of the three-year monitoring trial indicated that nearly all supply chains exhibited a positive correlation, the highest being $r = 0.72$, $P<0.001$ (Table 3.4) between FB and DMC. Thus, fruit maturity contributes only in part to FB susceptibility, as other factors are also associated with it.

A fruit handler survey suggested that postharvest handling operations are inherent contributors to IDs, including with FB being observed in sea freight and not in air freight consignments (Section 3.3.2.1). The time from harvest to the export market was a maximum of 15 d for air freight, while it was 31 - 36 d for sea freight. Similarly, FB was observed in domestic supply chains handled by one ripening centre, while fruit handled by other ripening centres realised good quality. The grower survey also revealed that fruit in the library trays did not express IDs, whereas fruit transported to the market did (Section 3.3.2.1). Significant positive correlation ($r = 0.39$, $P<0.05$) between FB incidence and postharvest TTUs, and between FB incidence and time from packing to the VHT facility ($r = 0.49$, $P<0.01$) in 2021/22 (Table 3.5). This further suggests that fruit are exposed to stress during supply chain distribution which increases expression of IDs. Thus. postharvest handling operations can have disproportionally more influence the preharvest ones in terms of fruit expression of FB.

The monitoring trial in 2021/2022 also revealed a positive correlation between shelf-life and FB incidence (Table 3.4), with fruit taking longer to ripen having a higher incidence and severity of FB. Overall, apart from some supply chains, non-VHT fruit was found to have relatively higher FB than VHT fruit (Appendices 23, 24, and 25). The results suggest that FB browning is also associated with senescence / over-ripening. Several studies have also indicated that FB is the function of storage time (Hofman *et al.,* 2010; Miguel *et al.,* 2013; Brecht *et al.,* 2019; Costa *et al.,* 2021). However, Brecht *et al.,* (2020) and Brecht *et al.* (2012) reported that internal flesh discolouration / browning in mango developed as a result of chilling injury.

3.4.3 Plant nutrition

Significant variation in FCWP and FB incidence in the present study across different supply chains originating from the same block were observed over the three-year monitoring trial (Tables 3.1 and 3.2). Fruit originating from the same block may be assumed to have similar nutrient levels, albeit not tested, as they are grown in the same environment with the same management practices. In this scenario, the only difference was postharvest handling practices and the fruit maturity.

Anecdotally, it is often presumed that growers are applying too much N and not enough Ca during fruit growth and development. However, the survey revealed that the growers in the present study used a balance of fertilizers (Appendix 11), with adequate Ca during the early fruit growth and development phases. Despite this, the incidence of IDs was unacceptably high.

In addition, in blocks with higher and lower soil Ca levels (e.g., Block M38-2 at soil Ca of 2106 ppm and block M56-1 at soil Ca of 912 ppm) (Appendix 12), IDs did not differ significantly (Figures 3.3 and 3.4). This interpretation suggests by default that postharvest handling operations are prospectively more responsible for rendering fruit either more or less susceptible to express IDs rather than is nutritional balance.

While the working hypothesis of a role of nutrients in IDs for 'B74' mango was not affirmed, the possibility that fruit nutrition plays a role in making fruit robust and less susceptible to IDs. To conclusively determine the role of nutrient in the investigated disorders, further studies employing a wide range of Ca and N application rates and harvesting fruit at the same maturity will be necessary.

3.5 Conclusions

Two IDs in 'B74' supply chains were identified as being of economic importance, they are being defined as FCWP and FB with a view to avoid confusion with other specific disorders and / or generic terms like 'internal breakdown', 'internal flesh breakdown', and 'internal disorder'.

FCWP was associated with the mandatory VHT market-access treatment. FB was irrespective of ±VHT. Limited research has been directed towards understanding the underlying biology of, and factors contributing to, manifestation of such IDs, much less to establishing commercial control measures.

From this study, these specific disorders are complex and the result of interacting factors, rather than single cause. 'B74' fruit susceptibility to IDs varies with regions, blocks, and seasons, making it challenging to identify their specific causes.

This three-season study identified transit time (postharvest time), postharvest TTUs and harvest maturity as a potential factor that might contribute to IDs in 'B74' mango fruit. Maturity was identified as the most important factor, with FCWP negatively and FB positively correlated with it.

This study did not definitively affirm the hypothesis that VHT is the primary cause of FB disorder in 'B74' export supply chains. Similarly, the hypothesis of nutrient role in IDs could not established from this study. However, it can still be generally concluded that balanced nutrition can make the fruit more robust and tolerant to different stresses and disorders, even

though these elements may not be the primary causes of the IDs in question. Follow-on research and development could pursue the leads revealed in this study. This may include detailed fruit maturity coupled with postharvest TTUs experiments to build upon current findings gleaned over several harvest seasons.

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Chapter 4 Maturity and internal disorders of 'B74' mango fruit for export

4.1 Introduction

Tephritid fruit flies pose serious quarantine risk (Heather *et al.,* 1991). Freedom from infesting insects must be guaranteed for international trade with protocol countries. Postharvest heat disinfestation by vapour heat is a viable, non-chemical, and internationally acceptable control method for fruit flies in mango fruit (Joyce *et al.,* 1993; Jacobi *et al.,* 2001a). Vapour heat treatment (VHT) involves heating fruit with saturated hot vapour to a core temperature of 47 °C for 15 mins (Jacobi *et al.,* 2001a).

However, VHT can result in IDs (Esguerra *et al.,* 1990; Joyce *et al.,* 1993) characterised by cavities with white starchy tough lesions in the mesocarp (Esguerra *et al.,* 1990; Jha *et al.,* 2010); Chapter 1; Figure 1.5 B). In extreme conditions, VHT can damage fruit due to induced internal O_2 deficiency that results in fermentation (Esguerra *et al.,* 1990; Esguerrra and Lizada, 1990).

IDs in mango fruit are influenced by genetic, environmental, and management factors along with harvest maturity being pivotally important (Anwar and Malik, 2007). Relatively less mature mango fruit are prone to developing flesh cavity with white patches (FCWP) following hot water treatment (HWT) or VHT disinfestation (Brecht *et al.,* 2020; Khanal *et al.,* 2022). Immature 'Carabao' mango fruit were more susceptible to internal cavity and starchy lesions following VHT (fruit core temperature 46 °C for 10 mins) than were mature fruit (Esguerra *et al.,* 1990). Similarly, immature 'Kensington Pride' were more susceptible to FCWP following HWT (47 °C fruit core for 15 mins) than mature fruit (Jacobi *et al.,* 2001b).

In the above-mentioned studies, maturity assessment was subjective, making it difficult to identify sensitive fruit to IDs. Ideally, objective indicators of maturity stages sensitive to IDs are required. Dry matter content (represented as a percentage of dry weight to fresh weight; DMC) is one such objective indicator of maturity (Carvalho *et al.,* 2014). It has been used for maturity prediction in various fruit crops, including mango (Kinhal 2019).

A 24-h delay under typical ambient temperature before treatment (Brecht *et al.,* 2020) or a temperature conditioning treatment at 32 - 42 °C for 8 - 12 h before VHT / HWT helped reduce heat-induced disorders in mangoes (Klein and Lurie, 1992; Joyce *et al.,* 1993; Joyce and Shorter, 1994; Jacobi *et al.,* 1995; Jacobi *et al.,* 2001a; Brecht *et al.,* 2020), especially in

less mature fruit (Brecht *et al.,* 2020). Temperature conditioning involves pre-treating the product at an elevated temperature above the prescribed storage temperature for a specific duration (Jacobi *et al.,* 2001a).

Flesh browning (FB) is another important ID in 'B74' mango fruit (Chapter 1; Figure 1.5 A). It is thought to be associated with extended storage periods and / or significant delays between harvest and commencement of cold storage (Hofman *et al.,* 2010). Exact causes and mechanisms of FB in mango remain unresolved. However, FB in temperate fruits, such as apples and pears, has been studied extensively (Lau, 1998; Elgar *et al.,* 1999; Ferguson *et al.,* 1999; Franck *et al.,* 2007). It is influenced by combinations of specific pre- and post-harvest factors (Franck *et al.,* 2007). FB has been linked with fruit maturity (viz., higher in more mature fruit) and storage conditions (i.e., longer storage and low O_2 with elevated temperature) (Elgar *et al.,* 1999; Lammertyn *et al.,* 2000; Franck *et al.,* 2007). Studies on the role of maturity and storage conditions in mango fruit are otherwise limited.

Likewise, limited information exists on influences of maturity on susceptibility of 'B74' mango fruit to IDs. Considering Chapters 2 and 3, it was hypothesised that 'B74' mango fruit sensitivity to IDs is largely a function of fruit maturity, with sensitivity to FCWP after VHT decreasing with more advanced fruit maturity, conversely, fruit sensitivity to FB increased with higher maturity irrespective of VHT. Several studies have explored the relationship between fruit maturity and IDs in mango fruit (Boag *et al.,* 1990; Jacobi *et al.,* 2001b; Jacobi *et al.,* 1995; Khanal *et al.,* 2022), offering this strategy to mitigate them.

4.2 Materials and methods

4.2.1 Experiment 1: Harvest maturity (DMC) effects on IDs of 'B74' mango fruit

4.2.1.1 Fruit source

Fruit were sourced from a commercial mango farm near Childers, Queensland, Australia (-25.14° S, 152.37° E) in 2022 and 2023. The 7-year old 'B74' mango trees were grafted onto 'Kensington Pride' rootstock. Tree spacing was 7 m between rows and 4 m within rows.

4.2.1.2 Fruit harvest and treatments

Fruit were harvested with short peduncles using hand-held secateurs and picking poles. They were classified into three maturity grades based on DMC: less mature: <15% DMC,

optimum maturity: 15 - 17% DMC, and more mature: >17% DMC (Table 4.1). Each treatment was replicated three times, with each replication consisting of a tray with 20 fruit. Fruit DMC was measured as in Chapter 3, Section 3.2.2.4. Fruit were placed in commercial fibreboard mango trays lined with a polypropylene insert. They were held in a vehicle overnight at ambient temperature (*ca.* 25 °C) and then transported over ~365 km for ~5 h in an air-conditioned vehicle to the postharvest laboratory at Gatton Campus within 20 h of harvest. They were held at ~16 °C overnight and de-stemmed and de-sapped in Mango-Plus® (580 g / kg alkaline salts and 500 ppm available chlorine active ingredients; Avanti Chemicals, Ormeau, Queensland) solution at 1 kg / 500 L of water for 90 seconds and then Sportak® (450g a.i. / L prochloraz; FCM Australasia Pty. Ltd., Murarrie, Queensland) fungicide at 55 ml / 100 L of water for 30 seconds. Fruit were then air-dried and held at ~16 °C overnight. Half of the fruit from each maturity class underwent VHT and the remainder as controls (Table 4.1). Next day they were transported by air-conditioned vehicle over ~72 km in ~ 1 h to a commercial VHT facility at Rocklea, Brisbane (-27.53° S, 152.99° E) for VHT. Control fruit were maintained at ~20 °C and ~90% RH at the laboratory.

Disinfestation treatment (VHT), fruit maturity, firmness, shelf-life, and IDs were assessed as outlined in Chapter 3, Sections 3.2.2.3, 3.2.2.4, and 3.2.2.5, respectively.

Table 4.1: Experiment 1 treatments to assess harvest maturity (DMC) effect on incidence and severity of flesh cavity with white patches (FCWP) in 'B74' mango fruit in 2022 and 2023 harvest seasons.

Maturity (DMC)	VHT
<15	+
15 - 17	+
>17	+
<15	-
15 - 17	-
>17	-

4.2.1.3 Starch iodine staining

Three fruit from each maturity class were randomly sampled after VHT. Fruit were longitudinally sliced on both sides of the seed. Lugol's solution (1 g potassium iodide plus 0.25

g iodine in 100 ml water) was applied to the fruit halves by paint brush and left for 30 seconds. Starch gives a black stain after Lugol's solution application (Reid *et al.*, 1982).

During IDs assessment, fruit with FCWP were stained with Lugol's solution to test that white patches in VHT-induced FCWP represented starchy lesions implying that disrupted starch hydrolysis is involved.

4.2.1.4 Gas exchange measurement

In 2022, three fruit from each treatment (Table 4.1) were sampled. After VHT, the fruit were transported to Maroochy Research Facility (MRF) over ~115 km in ~1.5 h in an air-conditioned vehicle and held at 16 °C for about 1 h to cool. They were then placed in 2 L polypropylene respiratory chambers and kept at a temperature of 20 °C and RH of >90%. Three additional chambers were kept empty to serve as reference for correction factors. Ethylene-free, humidified air was passed through the chambers at a target flow rate of 150 ml/min. Each chamber was sequentially connected via a Blue Box (ADAM - 4017; Utility Data Acquisition, Advantech America, Milpitas, CA, USA) sample stream selector to an infrared gas analyser (IRGA; HORIBA, PIR-2000). The IRGA continuously measured CO_2 concentration in gas streams quantified by a digital mass flow meter (DFM model DFM26, Aalborg®, Pryde Measurement Pty Ltd, Ashburton, Victoria, Australia). CO_2 concentration for each chamber was measured once every 3 h. Respiration rates were calculated by Smith (1993):

$$CO_2 \text{ production rate (mg/kg/hr)} = \frac{[(\%CO_2 - \text{Blank}\%CO_2) \times \text{Flow Rate(ml/min)} \times 321750])}{[\text{Wt} \times (273 + \text{Temp °C of gas flow})]}$$

Where, $\%CO_2$: CO_2 concentration measured for chamber with fruit.

Blank $\%CO_2$: CO_2 concentration measured for blank chamber.

Flow rate: measured flow rate of gases supplied in the chamber.

Wt: Fruit fresh weight (g).

In 2023, the experiment was conducted with minor modifications to methodology: viz., 2 L stainless steel chambers were utilised as respiratory chambers. Containers were changed because polypropylene chambers absorb ethylene gas. A food quality polycarbonate drain rack (Size 1/6; Cambro®) in the bottom of the respiratory chambers supported the fruit. Ethylene-free humidified air was passed through the chambers at a target flow rate of 120 ml / min. As mentioned above, CO_2 concentration was measured once every 3.5 h for each chamber. Fruit was sealed in the chamber for 6 h for ethylene accumulation and measurement. Prior to sealing the vessel, 5 ml potassium hydroxide solution (1 M KOH) was placed in a beaker with folded

filter paper (150 mm diameter papers, Westlab Pty Ltd, Victoria, Australia) to absorb CO_2. The beaker was placed inside the respiratory chamber along with the fruit. After 6 h the headspace gas was sampled by syringe (5 ml) through a silicon septum in the chamber lid. The gas samples were analysed for ethylene using a gas chromatograph (GC-2010 Plus, Shimadzu Scientific Instruments, Sydney, Australia). However, as no ethylene was detected over seven days, and as confirmed with Kitagawa® (concentration range 0.1 to 100 ppm; Kanagawa, Japan) ethylene gas detector tubes, monitoring for ethylene was discontinued.

4.2.1.5 Experiment design and data analysis

The experiment consisted of two factors, three maturity classes +/- VHT (Table 4.1). Replication was 3-fold and each replicate consisted of 20 fruit. Analysis of variance between treatments was performed and means separated by Tukey's HSD at $P<0.05$. In the case of +/- VHT, means were separated by *t*-test at $P<0.05$. Gas exchange data were subject to analysis of variance to assess variation in CO_2 production in fruit of different maturity +/- VHT on each individual day. Tukey's HSD test at $P<0.05$ was used to separate means. Data were analysed using JMP Pro 16.0.0. Images of starch-iodine-stained fruit were captured for relative comparison of starch hydrolysis level in fruit mesocarp.

4.2.2 Experiment 2: Effects of maturity stage on IDs of 'B74' mango fruit

4.2.2.1 Fruit source and treatments

This experiment was conducted in two harvest seasons (2022 and 2023). Fruit source was same as in Section 4.2.1.1. Two factors were tested in this experiment: fruit maturity stage, and conditioning treatment prior to VHT. In 2022, fruit were harvested at two maturity stages i.e., early (1-week before commercial harvest), and late (1-week after commercial harvest) over a 2-week time (Table 4.2). In 2023, fruit were harvested at three maturity stages i.e., early (2-week before commercial harvest), mid (commercial harvest), and late harvest (2-week after commercial harvest) over a month time (Table 4.2). Fruit were handled as in Section 4.2.1.2. Fruit were then placed at ~16 °C, except those subjected to conditioning treatment which were stored at 37 ± 1 °C for 12 h. The treatments imposed are shown in Table 4.2. The fruit were then transported in an air-conditioned vehicle over ~72 km in ~ 1 h to a commercial VHT facility at Rocklea, Brisbane for VHT. The untreated controls were left in the shelf-life room where temperature and humidity were maintained at ~20 °C and ~90%, respectively.

Disinfestation treatment, maturity, firmness, shelf-life, and IDs assessment were as described in Chapter 3, Sections 3.2.2.3, 3.2.2.4, and 3.2.2.5, respectively.

Table 4.2: Experiment 2 treatments to assess maturity stages and the temperature conditioning effects on the incidence and severity of flesh cavity with white patches (FCWP) and flesh browning (FB) in 'B74' mango fruit in 2022 and 2023. In 2022, only early (1-week before commercial harvest) and late harvests (1-week after commercial harvest) were conducted, whereas in 2023, three harvests i.e., early (2-week before commercial harvest), mid (commercial harvest), and late (2-week after commercial harvest) were done.

Harvest	Conditioning at 37 ± 1 °C for 12 h	VHT	Harvest year
Early	-	-	**2022/23**
Early	-	+	**2022/23**
Early	+	+	**2022/23**
Mid	-	-	**2023**
Mid	-	+	**2023**
Mid	+	+	**2023**
Late	-	-	**2022/23**
Late	-	+	**2022/23**
Late	+	+	**2022/23**

4.2.2.2 Starch iodine staining

Before taking fruit to the VHT, three fruit from each conditioned and non-conditioned treatment were randomly selected and treated with Lugol's solution (Plant Essentials, Deeragun, Qld, Australia) as in Section 4.2.1.3. This was done to observe the effect of conditioning in starch hydrolysis prior to VHT.

4.2.2.3 Experiment design and data analysis

The experiment consisted of six and nine treatments in 2022 and 2023, respectively (Table 4.2) and each treatment was replicated three folds and each replicate tray contained 20 fruit. Analysis of variance between the treatments was done and the means were separated by Tukey's HSD at $P<0.05$. In 2022, there were only two harvest, therefore the means were separated by *t*-test at $P<0.05$. Data were analysed using JMP Pro 16.0.0.

4.2.3 Experiment 3: Effect of fruit maturity (determined by floatation technique) on IDs of 'B74' mango fruit with controlled atmosphere storage.

4.2.3.1 Fruit source and treatments

Fruit were sourced as in Section 4.2.1.1. Fruit were sorted into two maturity classes based on a floatation technique (Kapse and Katrodia, 1996). Table salt (Saxa iodised table salt; distributed by Salpak Pty Ltd, Seven Hills, New South Wales) 500 g was dissolved in 25 L of water (at 25 °C) i.e., 2% salt solution in 2022 and 300 g in 24 L of water (at 25 °C) i.e., 1.25% salt solution in 2023. The reason for the disparity in salt concentration between the two years was to achieve a balance of approximately 50% floaters and 50% sinkers. Fruit were harvested as in Section 4.2.1.2 and placed into the salt solution. Floaters were considered less mature and sinkers were considered more mature (Kapse and Katrodia, 1996). Fruit were held overnight and transported the next day in an air-conditioned car to the MRF (~213 km; ~3 h). Fruit were de-stemmed, de-sapped, and fungicide treated as in Section 4.2.1.2. Fruit were then air-dried, and held at ~16 °C overnight. Next day they were transported by air-conditioned vehicle over ~72 km in ~1 h to a commercial VHT facility at Rocklea, Brisbane for VHT. VHT was performed as in Chapter 3, Section 3.2.2.3, after holding fruit for 21 h in ambient condition. Post-VHT, fruit were transported to the MRF in an air-conditioned car and placed at ~16 °C overnight. After an initial fruit DMC assessment as in Chapter 3, Section 3.2.2.4, fruit from each maturity class were stored at two different conditions i.e., controlled atmosphere (CA; Temperature 11 °C; gas composition 3% O_2 and 5% CO_2), and the controls (Temperature: 11 °C; ambient atmosphere) for 21 days (Table 4.3). After this period, fruit were placed in a shelf-life room at ~20 °C and ~90% RH till the eating soft stage. IDs assessment was done as described in Chapter 3, Section 3.2.2.5.

Table 4.3: Treatments to assess maturity (determined by floatation technique) effect on flesh cavity with white patches (FCWP) and flesh browning (FB) incidence and severity in 'B74' mango fruit in 2022 and 2023.

Maturity	VHT	Storage for 21 days at 11 °C
Floating	+	CA (3% O_2 and 5% CO_2)
Floating	+	Control (ambient atmosphere)
Sinking	+	CA (3% O_2 and 5% CO_2)
Sinking	+	Control (ambient atmosphere)

4.2.3.2 Experiment design and data analysis

The experiment consisted of two levels of maturity and two storage conditions (Table 4.3). Each treatment was replicated three-fold, and each replicate tray contained 15 fruit. Analysis of variance between treatments was conducted and means separated by *t*-test at *P<0.05*. Data were analysed using JMP Pro 16.0.0.

4.3 Results

4.3.1 Experiment 1: Harvest maturity (DMC) effects on IDs of 'B74'mango fruit

4.3.1.1 Incidence and severity of internal disorders

In the 2022 season, maturity effects on FCWP incidence and severity were significant *(P<0.05)* (Appendices 26 and 27). FCWP was observed only in VHT fruit. VHT fruit with <15% DMC had a significantly *(P<0.05)* higher FCWP incidence at 24.6% and severity at 0.4 (Table 4.4). However, FCWP incidence and severity in VHT fruit with >15% DMC were similar *(P>0.05)* to untreated controls (Table 4.4). FB was not observed in this 2022 experiment, and no disorders were observed in 2023.

Table 4.4: Maturity (DMC), vapour heat treatment (VHT), and interactive effects on incidence and severity (mean ± SE) of flesh cavity with white patches (FCWP) in 2022. Treatment effect was assessed by analysis of variance and means separated by Tukey's HSD at *P<0.05*.

Maturity (DMC)		FCWP incidence (%)	FCWP severity (0 - 3)
<15 (n = 60)		12.4 ± 5.7^{a}	0.2 ± 0.1^{a}
15 - 17 (n = 60)		3.9 ± 2.6^{b}	0.1 ± 0.1^{b}
>17 (n = 60)		2.4 ± 2.4^{b}	0.1 ± 0.1^{b}
Treatment			
Non-VHT (n = 90)		0.0 ± 0.0^{b}	0.0 ± 0.0^{b}
VHT (n = 90)		12.4 ± 3.7^{a}	0.2 ± 0.1^{a}
Treatment × Maturity (DMC)			
VHT	<15 (n = 30)	24.6 ± 2.4^{a}	0.4 ± 0.1^{a}
	15 - 17 (n = 30)	7.7 ± 4.4^{b}	0.1 ± 0.0^{ab}
	>17 (n = 30)	4.8 ± 4.8^{b}	0.1 ± 0.1^{b}
Non-VHT	<15 (n = 30)	0.0 ±0.0 ^{b}	0.0 ± 0.0^{b}
	15 - 17 (n = 30)	0.0 ±0.0^{b}	0.0 ± 0.0^{b}

>17 (n = 30)	0.0 ±0.0^{b}	0.0 ± 0.0^{b}

Mean values represented by same letter(s) are not significantly *(P<0.05)* different.

4.3.1.2 Respiration rate

During the 2022 season, no differences *(P<0.05)* in rates of CO_2 production for 10 days across different maturity fruit +/- VHT (Figure 4.1). CO_2 production in all fruit steadily increased from day 0, reaching a peak on day 5 before decreasing thereafter. In the 2023 harvest season, no significant difference *(P<0.05)* was discerned in CO_2 production on day 0 among different maturity class fruit +/- VHT (Figure 4.2). However, significant differences *(P<0.05)* were observed in CO_2 production on days 1, 2, and 3 (Appendices 35, 36, and 37). After day 4, there were no significant differences *(P<0.05)* in CO_2 production among treatments to day 13. More and optimum maturity fruit reached climacteric peaks on day 4, while the less mature fruit climaxed on day 5. After day 4 in more and optimum maturity fruit and after day 5 in less mature fruit, CO_2 production decreased more-or-less steadily to end of experiment (Figure 4.2).

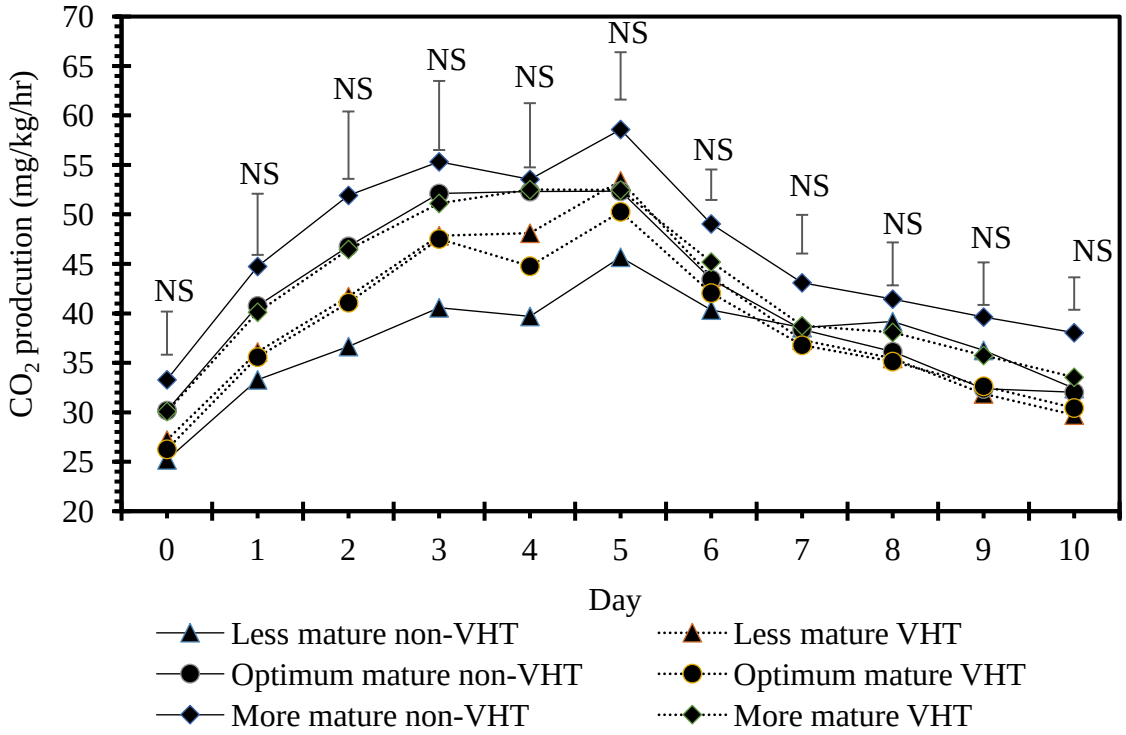


Figure 4.1: CO_2 production of different mature i.e., less (<15% DMC), optimum (15 - 17% DMC) and more (>17% DMC) mature 'B74' mango fruit +/- vapour heat treatment (VHT) during 2022 harvest season. Each point is the average of three replicate fruit. The vertical bar indicates the standard error of mean difference. NS: not significant.

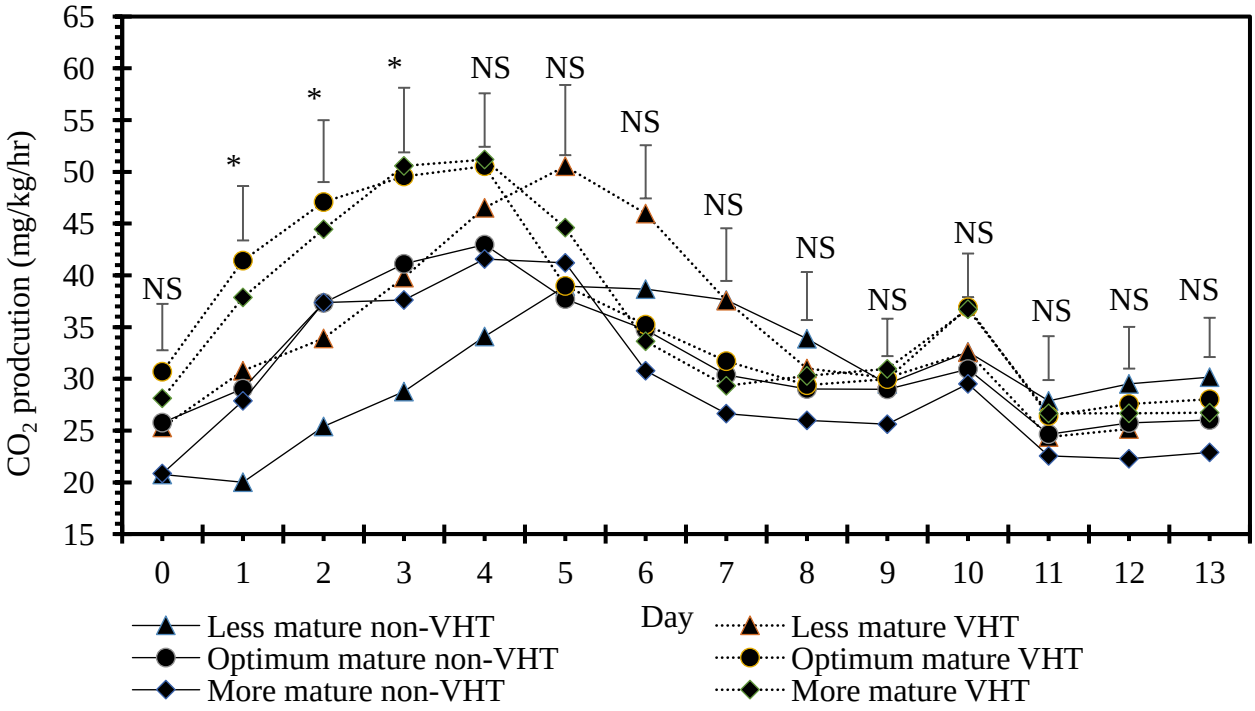


Figure 4.2: CO_2 production of different mature i.e., less (<15% DMC), optimum (15 - 17% DMC) and more (>17% DMC) mature 'B74' mango fruit +/- vapour heat treatment (VHT) during 2023 harvest season. Each point is the average of three replicate fruit. The vertical bar indicates the standard error of mean difference. Significance codes *: 0.05, NS: not significant.

4.3.1.3 Starch iodine staining

Figure 4.3 shows starch iodine staining of the different maturity fruit after VHT. In more mature fruit, starch hydrolysis had commenced in the inner mesocarp, near the seed and where the FCWP is typically observed (Figure 4.3). In less mature fruit, starch hydrolysis hadn't progressed appreciably. That white lesions within the mesocarp (Figure 4.4; left) are starchy was confirmed by its black staining by iodine (Figure 4.4; right). Hydrolysis of starch was disrupted by VHT.



Figure 4.3: Starch iodine staining for starch after VHT in less mature (left), optimum mature (middle), and more mature (right) fruit. Starch stains black.



Figure 4.4: Starch iodine staining affirming disruption of starch hydrolysis in fruit expressing flesh cavity with white patches. White lesions in flesh after VHT (left) are starchy as confirmed by black starch-iodine staining (right).

4.3.2 Experiment 2: Effects of maturity stages on IDs of 'B74' mango fruit

4.3.2.1 Fruit maturity

Average DMC was 14.5% (range 12.2 - 17.4%) and 17.4% (range 14.6 - 21.2%) for early- and late-harvested fruit, respectively, in the 2022 season. In the 2023 season, average DMC was 14.5% (range 11.6 - 18.6%), 16.1% (range 14.2 - 18.7%), and 18.1 % (range 14.3 - 22.9%) for early, commercial, and late harvest maturity fruit, respectively.

4.3.2.2 Incidence and severity of internal disorders

Maturity stages significantly *(P<0.05)* affected the incidence and severity of FCWP (Table 4.5; Appendices 28 and 29). Late-harvested fruit showed 70% reduction of FCWP versus early harvested fruit. Like previous experiments (Section 4.3.1.1), untreated fruit had no FCWP. Fruit conditioned at 37 ± 1 °C for 12 h prior to VHT had significantly *(P<0.05)* reduced FCWP disorder (Table 4.5), with a seven-fold reduction in early harvest compared to four-fold reduction in late harvest. FB (2.8%) was observed only in late harvested fruit, with an insignificant *(P<0.05)* average severity of 0.02 (Appendices 30 and 31). No disorders were observed in the repeat 2023 harvest season trial.

Table 4.5: Fruit maturity, vapour heat treatment (VHT) with and without temperature preconditioning at 37 ± 1 °C, and interactive influence on incidence and severity (mean ± SE) of flesh cavity with white patches (FCWP) and flesh browning (FB) in the 2022 harvest season. Analysis of variance between treatments was assessed and significant means were separated by Tukey's HSD at *P<0.05*.

Harvest / maturity		FCWP incidence	FCWP severity	FB incidence	FB severity
Early (14.5% DMC; n = 60)		26.9 ± 11.2[a]	0.3 ± 0.1[a]	0.0 ± 0.0	0.0 ± 0.0
Late (17.4% DMC; n = 60)		8.3 ± 3.4[b]	0.1 ± 0.1[b]	2.9 ± 1.5	0.1 ± 0.1
Treatments					
Non-VHT (n = 60)		0.0 ± 0.0[b]	0.0 ± 0.0[b]	1.7 ± 1.7	0.0 ± 0.0
VHT (n = 60)		45.0 ± 11.9[a]	0.5 ± 0.2[a]	1.7 ± 1.7	0.0 ± 0.0
Conditioning + VHT		7.8 ± 3.0[b]	0.1 ± 0.0[b]	0.8 ± 0.8	0.0 ± 0.0
Harvest × Treatments					
Early harvest	Non-VHT	0.0 ± 0.0[c]	0.0 ± 0.0[b]	0.0 ± 0.0	0.0 ± 0.0
	VHT	70.0 ± 7.6[a]	0.8 ± 0.1[a]	0.0 ± 0.0	0.0 ± 0.0
	Conditioning + VHT	10.6 ± 5.2[bc]	0.1 ± 0.1[b]	0.0 ± 0.0	0.0 ± 0.0
Late harvest	Non-VHT	0.0 ± 0.0[c]	0.0 ± 0.0[b]	3.3 ± 3.3	0.0 ± 0.0
	VHT	20.0 ± 5.0[b]	0.2 ± 0.0[b]	3.3 ± 3.3	0.0 ± 0.0
	Conditioning + VHT	5.0 ± 2.9[c]	0.03 ± 0.0[b]	1.7 ± 1.7	0.0 ± 0.0

Mean values represented by same letter(s) are not significantly *(P<0.05)* different.

4.3.2.3 Starch iodine staining

Initiation of starch hydrolysis was confirmed by starch iodine staining of fruit exposed to conditioning treatment. Non-conditioned fruit had black stain in the whole flesh, signifying that no starch hydrolysis had initiated, whereas conditioned fruit had no black stain in the centre of the fruit (~15% of fruit flesh), signifying that starch hydrolysis had initiated (Figure 4.5).



Figure 4.5: Starch iodine staining for starch in mango fruit flesh with (right) and without (left) temperature conditioning of 37 ± 1 °C prior to vapour heat treatment (VHT).

4.3.3 Experiment 3: Effect of fruit maturity (determined by floatation technique) on IDs of 'B74' mango fruit

4.3.3.1 Fruit maturity

The average DMC of the floaters and sinkers were 15.3% and 15.9%, respectively in 2022, and 15.5% and 15.5%, in 2023, respectively and they were not different *(P>0.05)*.

4.3.3.2 Incidence and severity of disorders

In 2022, effect of fruit maturity classes sorted by floatation technique on the incidence and severity of FCWP and FB were not significant *(P<0.05)* (Appendices 32 and 33). In 2023, FCWP was not observed in this experiment. Storage conditions had significant effects *(P<0.05)* on the incidence and severity of FB (Table 4.6; Appendices 34 and 35). CA significantly *(P<0.05)* reduced FB incidence (33.3%) and severity (0.3) from 71.1% incidence and 0.8 severity in ambient storage. There was no interaction *(P<0.05)* between maturity and storage conditions on the incidence and severity of FB (Table 4.6). These findings were consistent with those observed in the 2023 trial.

Table 4.6: Fruit maturity (sorted by floatation) effect on FB incidence and severity (mean ± SE) on 'B74' mango fruit in 2022 and 2023. Maturity was sorted by floatation in 2% and 1.5% salt solution in 2022 and 2023, respectively. Analysis of variance between treatments was assessed and means were separated by *t*-test at *P<0.05*.

	2022		2023	
Maturity	FB severity	FB incidence	FB severity	FB incidence
Floating (n = 90)	0.6 ± 0.2	57.8 ± 11.4	0.4 ± 0.2	32.2 ± 8.7
Sinking (n = 90)	0.5 ± 0.1	46.7 ± 8.4	0.4 ± 0.2	32.2 ± 11.6
Storage conditions				
Control (n = 90)	0.8 ± 0.1[a]	71.1 ± 5.1[a]	0.71 ± 0.0[a]	53.3 ± 3.3[a]
CA (n = 90)	0.3 ± 0.1[b]	33.3 ± 6.7[b]	0.08 ± 0.1[b]	11.1 ± 4.6[b]
Maturity × storage conditions	NS	NS	NS	NS

Mean values represented by same letter(s) are not significantly *(P<0.05)* different. NS: non-significant.

4.4 Discussion

4.4.1 Flesh cavity with white patches

These results indicated that less mature fruit (<15% DMC) were more susceptible to FCWP after VHT (Tables 4.4 and 4.5). Likewise, Jacobi *et al.* (2001b) reported that less mature 'Kensington Pride' mango fruit were more prone to starchy layers and spots in the mesocarp, and tolerance to HWT-induced disorders increased as maturity increased. Similarly, Esguerra *et al.* (1990), Jacobi *et al.* (2001a), and Brecht *et al.* (2020) reported that less mature mango fruit were prone to cavity formation after VHT / HWT.

White starchy layers or spots in VHT fruit flesh may be attributable to disruption of starch hydrolysis due to heat. Enzymes that convert starch into sugar may be heat-sensitive (Joyce *et al.*, 1993). Disrupted starch degradation would thereby leads white starchy regions in the flesh. This outcome was substantiated through starch iodine staining. White regions exhibited higher starch levels, as indicated by staining with Lugol's solution (Figure 4.4, right). Fuchs *et al.*, (1980) found a significant increase in alpha-amylase activity in mango fruit from the 6th day of harvest, peaking on the 8th day post-harvest. Peak alpha-amylase activity occurs earlier in mature fruit than in less mature mango fruit. Acceleration of ripening processes in mature fruit may produce enough sugar, particularly sucrose, in advance heat treatment to

reduce injury from heat treatment (Jacobi *et al.*, 2001b). Accelerated starch degradation in more mature fruit than less mature fruit was shown by the starch iodine staining after the VHT (Figure 4.3). Evidence of protective role of sugar in different stress is described in Section 4.4.3.

Generally, injuries caused by VHT occur when duration and / or temperature of heat treatment surpass recommended guidelines (Brecht *et al.*, 2020). Towards avoiding VHT induced injuries, it is important to monitor and manage the treatment process, as followed by prompt cooling. To prevent excessive water loss, which can be exacerbated by heat treatment, it is recommended to maintain a relative humidity of 90 - 95% and / or utilize plastic film liners or bags to protect the fruit (Brecht *et al.*, 2020).

The process leading to cavity formation in heat-treated mango is unclear. Heat treatment disrupts cellular integrity and reducs cell-to-cell adhesion. As surrounding tissue ripens, starch is converted into sugar, osmotically drawing water away from affected regions (Joyce et al., 1993). Specific regions of the flesh damaged by heat may undergo dehydration, and desiccated mesocarp tissue may collapse, resulting in cavities within the flesh (Joyce *et al.*, 1993).

4.4.2 Flesh browning

From this experiment 3 above, FB in mango fruit was associated with storage conditions (Table 4.6). For example, fruit in experiment 1, 2, and 3 were sourced from the same farm and block and handled in the same way as much as possible before allocation to treatments. However, very high FB incidence was observed only in experiment 3 (Table 4.6). In experiments 1 and 2, fruit were held at 20 - 22 °C and >95% RH immediately post-VHT for ripening (Sections 4.2.1.2 and 4.3.2.1). In contrast, in experiment 3 and post-VHT, the fruit were held at 16 °C overnight, followed by storage at 11 °C with and without CA for 21 days (Section 4.2.3.1). The fruit were then removed from storage conditions and held at 20 - 22 °C and >95% RH until eating ripe stage. Given that fruit stored for 21 days in CA and ambient conditions only had FB, suggest that FB is associated with long storage time (Table 4.6). Other studies also suggest that FB is a function of storage time and temperature (Hofman *et al.*, 2010; Brecht *et al.*, 2012; Miguel *et al.*, 2013; Brecht *et al.*, 2019; Brecht *et al.*, 2020). Longer storage durations can increase polyphenol oxidase activity in mango fruit, suggesting intensification of

oxidative processes (Miguel *et al.,* 2013). Costa *et al.* (2021) reported that FB index in 'Palmer' mangoes increased from 41.2 after 7 days to 79.3 after 28 days of storage at 16 °C.

FB in mangoes may be attributed to over-ripening (Gabriëls *et al.,* 2020). Ripening involves alterations in the physiochemical properties of the cell wall, resulting in compositional and structural changes (Posé *et al.,* 2019). However, when fruit over-ripen, cell senescence may manifest as tissue browning. More mature fruits were more susceptible to FB disorder, possibly because diffusivity to gases reduces as fruit matured and ripen (Crisosto, 1995). Changes in the microstructure of fruit tissue during ripening include decreasing pore size, increasing pore fragmentation and increasing pore specific surface area, and concomitant effect on respiratory metabolism (Cantre *et al.,* 2020). Reduction in the ability of O_2 to diffuse into the fruit would result in localised O_2 starvation and build-up of CO_2 in the inner mesocarp and lead to fermentation (Crisosto, 1995).

4.4.3 Temperature conditioning

Fruit conditioned at 37 ± 1 °C for 12 h prior to VHT had significantly *($P<0.05$)* reduced FCWP disorder (Table 4.5). Unconditioned VHT treated fruit had 45.0% FCWP incidence, while conditioned fruit had only 7.8% FCWP. Jacobi *et al.* (2001a) and Klein and Lurie (1992) also reported that conditioning before heat treatment protects fruit from stress injury and physiological disorders. Compared to maintaining fruit at ambient temperatures, HWT-treated mango fruit stored at 13 °C between harvest and treatment became unmarketable (Smith and Chin, 1989).

Warm temperature conditioning affects fruit physiology. For instance, acceleration of ripening associated starch degradation with concomitant accumulation of sucrose. Starch iodine staining confirmed that conditioning treatment led to starch hydrolysis initiation in fruit. Non-conditioned fruit showed black stain throughout the mesocarp, while conditioned fruit had an unstained inner mesocarp (Figure 4.5). Researchers have also reported that partially ripe mango fruit is relatively more tolerant physiological disorders including chilling injury (Mohammed and Brecht, 2002), under skin browning (Marques *et al.,* 2012; Marques *et al.,* 2014), and irradiation damage (Boag *et al.,* 1990; Sultana *et al.,* 2021). Collectively these studies show that elevated sugar content is important in enhanced fruit tolerance to stress. Sucrose protects cells from osmotic stress by stabilizing cellular structures and maintaining turgor pressure.

Overall, time delay or conditioning prior to heat treatment should be more beneficial for less mature fruit (Brecht *et al.*, 2020). Nevertheless, pre-conditioning prior to VHT may not eliminate FCWP disorder. In the present study, conditioned fruit had seven-fold in early harvested compared to four-fold reductions in late harvested fruit, respectively (Table 4.5). Relatively greater susceptibility in less mature fruit might be because more mature fruit have expressed enzymes for starch degradation (e.g., alpha amylase). Conversely, activation of these enzymes in relatively immature fruit might not have progressed much before heat treatment that could inhibit their activity and contribute the starchy layers observed.

However, for effective commercial phytosanitary treatment, it is that treatments meet quarantine restrictions. In this regard, enhancing thermotolerance of fruit through temperature conditioning may also enhance the thermotolerance of targeted insect pests (Beckett and Evans, 1997). Beckett and Evans (1997) noted that conditioning led to an increased time required for conditioned *B. tryoni* larvae and eggs to reach mortality levels equivalent to those of non-conditioned larvae. Moreover, the efficacy of conditioning toward providing protection to fruit depends on a number of factors, including the season, fruit ripeness, pre-treatments, and temperatures (Paull and Armstrong, 1994).

4.5 Conclusions

FCWP and FB are the major IDs observed in this study. FCWP was induced by VHT, a mandatory disinfestation treatment for fruit export. The treatment window between successful disinfestation and loss of fruit quality is narrow and so sometimes leads to heat induced internal flesh injury.

VHT had differential effects on the fruit of varying maturity. Less mature fruit (<15% DMC) were relatively more sensitive to FCWP after VHT. Temperature conditioning prior to VHT ameliorated heat injury but did not eliminate it. Overall, physiological stage or age of fruit at time of treatment affects fruit sensitivity to VHT injury. Conditioning is relatively more beneficial for less mature fruit.

FB was observed in mango fruit at the end of shelf-life and the relationship with fruit maturity could not be established in this study. FB in mango fruit was a function of postharvest time and temperature. The risk of the FB disorder increased for fruit exposed to long supply chains. Further research into factors and their levels in predisposing fruit to FB is warranted to

better understand the causal mechanism. Maintaining optimum temperature and / or short supply chains help to assure reduced incidence of FB.

Moreover, other yet undefined factors might also be involved in fruit tolerance of or sensitivity to both FCWP and FB disorders. Further research is warranted to fully scope causal agents and their relative degree of influence. More comprehensive characterisation and quantification of positive and negative effectors and their interplay could inform better practices for supply of best consumer quality 'B74' fruit in export markets.

4.6 References

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Chapter 5 Spatial and temporal physiochemical changes in 'B74' mango fruit mesocarp during ripening after vapour heat treatment

5.1 Introduction

Fruit ripening is genetically regulated and involves physiological, biochemical, and organoleptic changes (Lizada, 1993). These include conversion of starch to sugars, decrease in acidity (Joyce *et al.,* 2002), loss of firmness, alterations in cell wall structure and composition, and reductions in structural integrity and intracellular space or mesocarp porosity (Mitra and Baldwin, 1997; Narain *et al.,* 1998; Tharanathan *et al.,* 2006; Cantre *et al.,* 2020; Gabriëls *et al.,* 2020).

Arrangements of cells and intercellular spaces within fruit influences the movement of respiratory O_2 and CO_2 gases (Herremans *et al.,* 2014). ATP generated through aerobic respiration is necessary for tissue maintenance and repair and ripening processes. Decrease in intercellular spaces at the microstructural level of fruit tissue during ripening influences respiratory metabolism (Cantre *et al.,* 2020) and can reduce available O_2 for respiration, leading to less energy efficient anaerobic fermentation and undesirable ethanol and acetaldehyde metabolites (Hernández-Sánchez *et al.,* 2007). Moreover, under low O_2 conditions, reactive O_2 species associated with metabolic stress can damage cell membranes (Streif *et al.,* 2001; Franck *et al.,* 2007). These maladies can result in breakdown of cells leading to tissue browning and collapse (Herremans *et al.,* 2014).

Such physiochemical changes can create spatial gradients within the mango mesocarp (Joyce *et al.,* 2002). For example, the inner mesocarp of 'Carabao' (Lizada, 1993) and 'Kensington Pride' (Joyce *et al.,* 2002) mango fruit softens prior relatively more than the outer mesocarp during fruit ripening. Spatiotemporal variations in the mesocarp are reflected in non-uniform water activity (Joyce *et al.,* 2002).

Understanding changes that occur during mango ripening is informative for post-harvest handling of mangoes. Magnetic resonance imaging (MRI) and X-ray µCT are non-destructive techniques that can visualise structural, water activity, and water mobility changes *in vivo* during ripening (Joyce *et al.,* 2002; Lammertyn *et al.,* 2003b; Defraeye *et al.,* 2013; Suchanek *et al.,* 2017; Zhou *et al.,* 2019). These imaging techniques can contribute to better understanding physiochemical and spatiotemporal processes associated with ripening (Chen *et al.,* 1989; Clark *et al.,* 1997; Butz *et al.,* 2005). Moreover, their non-destructive nature allows

repeated measurements on individual fruit throughout the process. MRI (Appendix 4) and X-ray µCT (Appendix 3) have been gainfully employed to study the internal structure of various fruits and vegetables.

The physiological, biochemical, and compositional changes during mango fruit ripening are documented. However, there is limited exploration of spatial and temporal physiochemical changes in mango flesh regarding fruit fly phytosanitary vapour heat treatment (VHT) effects on mango ripening. This preliminary study sought to utilise MRI and X-ray µCT to characterise physiochemical changes in mango mesocarp post VHT throughout ripening from hard green mature to soft ripe stage.

5.2 Methodology

5.2.1 MRI study

5.2.1.1 Fruit source

Six 'B74' hard green mature mango fruit at each of the three maturity stages, viz., 'less mature' at <15% dry matter content (represented as a percentage of dry weight to fresh weight; DMC), 'optimum mature' at 15 - 17% DMC, and 'more mature' at >17% DMC were utilised. They were of uniform size and weight, and free from defects and / or diseases. These fruit were freshly harvested from a commercial mango orchard near Childers, Queensland. Half from each maturity class were subjected to VHT and the other half kept as untreated controls. Fruit DMC was measured as per Chapter 3, Section 3.2.2.4. They were handled as in Chapter 4, Section 4.2.1.2.

5.2.1.2 Imaging

This study was conducted at the Centre for Advanced Imaging, University of Queensland, St Lucia. It compared and contrasted spatial and temporal physiochemical changes in different maturity fruit mesocarp tissue with and without VHT. A Siemens Magnetom Prisma 3T with a resolution of 256 × 230 matrices was used for MRI using a gradient echo sequence and 4 msec echo time. Slice thickness was 7 mm for nine slices, repetition time was 8.6 msec, and field strength was 2.9 T and field of view was 500 mm. When not being imaged, fruit were held in the facility at ~20 °C. Treated fruit were subjected to proton density (PD) and T2 imaging (considered as day 0) the day following VHT. Subsequent imaging was every third day thereafter (viz., days 3, 6, 9, and 12) up to 13 days post VHT.

5.2.1.3 MRI

Images obtained were viewed using advanced MicroDicom DICOM Viewer version 2023.1.1 (Build 1543) 64 bit. Regions of interest (ROI) were selected in outer, middle, and inner mesocarp tissues on both sides of the central seed (Figure 5.1). T2 and PD values were obtained for each of the three-replicate fruit (Figure 5.1). The T2 and PD values obtained were subject to analysis of variance for maturity and VHT treatments on inner, middle, and outer mesocarp tissues for serial ripening stages. Means were separated by Tukey's HSD at $P<0.05$. The values were plotted in graphs and images visually compared for fruit maturity fruit with and without VHT at successive ripening stages.

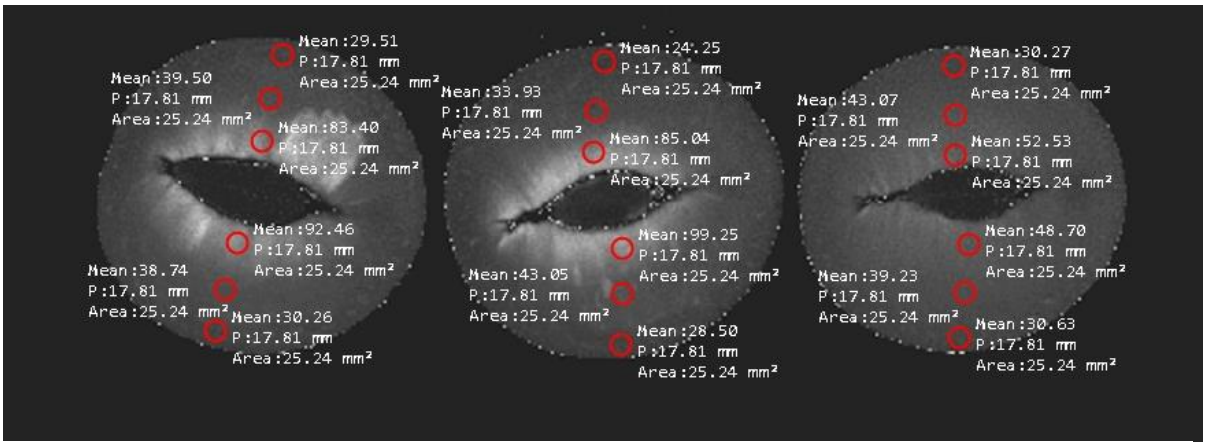


Figure 5.1: Region of interest (ROI) to assess spatiotemporal variation in physiochemical parameters of 'B74' mango flesh. Red circles (with area of 25.24 mm^2) represent ROIs in outer, middle, and inner mesocarp on both sides of the seed. Values were obtained as arbitrary units. MicroDicom DICOM Viewer version 2023.1.1 (Build 1543) 64 bit was used to collect these values.

5.2.2 X-ray µCT

5.2.2.1 Fruit source

Fruit source and handling were as per Section 5.2.1.1. Four randomly harvested 'B74' mango fruit were used.

5.2.2.2 X-ray µCT imaging

Three days after harvest, the fruit were subject to X-ray µCT imaging at the abovementioned Centre for Advanced Imaging.

5.2.2.3 Image analysis

CT images were viewed with MicroDicom DICOM Viewer version 2023.1.1 (Build 1543) 64 bit. Three ROIs for each of outer, middle, and inner mesocarp sections were selected (Figure 5.2). Values obtained were subject to analysis of variance between the different mesocarp areas. Means were separated by Tukey's HSD at $P<0.05$.



Figure 5.2: Region of interest (ROI) to assess the porosity across different sections of mesocarp. Red circle (with area of 25.10 mm^2) represent the ROIs in outer, middle, and inner mesocarp in both sides of seed along with the distal end from which the values were obtained. The MicroDicom DICOM Viewer version 2023.1.1 (Build 1543) 64 bit was used to obtain image values.

5.2.3 Fruit composition

In conjunction with the imaging analyses, a concurrent investigation into the composition of the fruit accompanied each imaging event. To complement the imaging, a parallel study of fruit composition reflected each imaging event. A total of 50 fruit for each maturity class (Section 5.2.1.1) were harvested. Fruit source was as mentioned in Section 5.2.1.1. Half were sent for VHT and the reminder held as untreated controls. Fruit were handled as per Chapter 4, Section 4.2.1.2. They were held at ~20 °C. At each imaging event, five fruit from each maturity - and + VHT were assessed for firmness, total soluble solids (TSS), and starch iodine staining. The data were subject to analysis of variance. Their purpose was to inform image interpretation.

5.2.3.1 Fruit firmness

At each imaging event, five fruit were randomly selected for the objective firmness assessment using a Fruit firm (TR, Turoni, Forli, Italy), a non-destructive digital firmness tester (Wijewardane, 2014). Four measurements were taken on each fruit with an average value being

recorded. The sites of four measurements were taken in such a way that it represented the whole fruit (Figure 5.3).

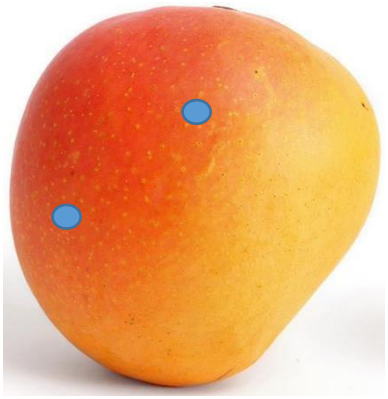


Figure 5.3: Positions of firmness measurement (blue mark) on the fruit. Opposite measurements were taken on the other side of the fruit.

5.2.3.2 Total soluble solids (TSS)

At each imaging event, three fruit were randomly selected for TSS measurement. Fruit were sliced longitudinally on both 'cheek' sides close to the seed. Juice from each slice onto the prism of a digital pocket refractometer (Atago PAL-1™, Japan) and the values recorded.

5.2.3.3 Starch iodine staining

For each imaging session, two fruit from each maturity class with and without VHT were chosen at random for starch iodine staining (Chapter 4, Section 4.2.1.3). On day 6, nearly all the starch had undergone hydrolysis, so starch staining was discontinued.

5.3 Results

5.3.1 MRI

5.3.1.1 T2 images

T2 value initially increased in all maturity fruit in all parts of the mesocarp, followed by a subsequent decrease (Appendix 48). The exception was non-VHT more-mature fruit, wherein the middle mesocarp exhibited a consistent decline in signal. In the inner mesocarp, a sharp increase in T2 value at day 3 was followed by a sharp decrease by day 6, followed by steady decrease to end of shelf-life. However, middle, and outer mesocarp did not show significant fluctuation in T2. Notably, inner mesocarp of non-VHT fruit demonstrated sharper increase in T2 value compared to VHT fruit. Less mature VHT fruit showed smaller changes in T2 values across the mesocarp regions compared to less mature non-VHT, optimum and more mature with and without VHT. Outer and middle mesocarp tissue exhibited minimal changes in T2 values (Appendix 48).

Statistical analysis revealed differences ($P<0.05$; Table 5.1; Appendix 40) in T2 values among different parts of the mesocarp at day 0. Specifically, the inner mesocarp exhibited

higher T2 values *(P<0.05)* than the middle and outer mesocarp. This trend persisted until the day 9. However, T2 values in different parts of the mesocarp were similar *(P>0.05)* on day 12. The effect of VHT on T2 values was significant (*P<0.05*; Table 5.1; Appendix 40), with non-VHT fruit exhibiting higher T2 values than VHT fruit in all imaging events. T2 values of mature fruit were also significantly (*P<0.05*; Table 5.1; Appendix 40) different in the first two imaging events on days 0 and 3. On day 0, more mature fruit had higher T2 values *(P<0.05)*, they being similar to those for optimum mature fruit. Less mature fruit, on the other hand, had lower T2 values *(P<0.05)*, also similar to those of optimum mature fruit. On day 3, both more mature and optimum mature fruit had similar T2 values, while less mature fruit had lower T2 values *(P<0.05).* After day 6, T2 values were similar between different mature fruit.

Table 5.1: T2 values for 'B74' mango fruit mesocarp spatial (inner, middle, and outer mesocarp) and temporal (days after VHT until the end of shelf-life, i.e., 12 days) dimensions with and without vapour heat treatment (VHT) across fruit maturities; less: <15% DMC, optimum: 15 - 17% DMC, more: >17% DMC. Values were acquired individually for each of the three replicate fruits, and for each fruit, data points were collected from both cheeks.

Mesocarp position	Day 0	Day 3	Day 6	Day 9	Day 12
Inner	51.1[a]	71.0[a]	46.2[a]	38.4[a]	32.3
Middle	34.2[b]	40.8[b]	37.9[b]	30.3[b]	27.3
Outer	24.8[c]	30.2[c]	30.3[c]	27.8[b]	28.0
Treatment					
VHT	33.4[b]	43.4[b]	33.5[b]	28.4[b]	26.2[b]
Non-VHT	40.0[a]	51.3[a]	42.8[a]	35.9[a]	32.2[a]
Maturity					
More	41.0[a]	49.5[a]	37.8	32.5	30.7
Optimum	35.4[ab]	51.2[a]	40.0	33.0	28.8
Less	33.7[b]	41.4[b]	36.7	31.0	28.1

Mean values represented by same letter(s) are not significantly *(P<0.05)* different.

5.3.1.2 Proton density (PD) images

PD value at different stages of ripening did not vary much in each mesocarp section except in inner mesocarp of optimum mature VHT fruit (Appendix 49). However, PD imaging demonstrated significant variation in different parts of the mesocarp at different stages of ripening (*P<0.05*; Table 5.2; Appendix 41). On day 0, the inner mesocarp had higher PD values

(P<0.05) compared to the middle and outer mesocarp. Similarly, on day 3, the inner mesocarp had higher PD values *(P<0.05)*, which were similar to those of the middle mesocarp. This trend persisted in subsequent imaging events, where the inner mesocarp exhibited higher PD values *(P<0.05)* followed by the middle and outer mesocarp. The effect of VHT in the PD value was significant *(P<0.05)* on day 0 and 3 where VHT fruit had higher PD value than non-VHT fruit, thereafter the effect of VHT was not significant *(P>0.05)*. Effect of maturity in PD value was significant *(P<0.05)* till 9th day of imaging however, on day 12 the effect was not significant. More and optimum mature fruit had similar PD value but higher *(P<0.05)* than less mature fruit from day 0 to 9th day of imaging.

Table 5.2: PD value of 'B74' mango fruit mesocarp in spatial (inner, middle, and outer mesocarp) and temporal (days after VHT until the end of shelf-life i.e., 12 days) perspective, with and without vapour heat treatment (VHT), across the fruit of different maturity (less: <15% DMC, optimum: 15 - 17% DMC, more: >17% DMC). Values were acquired individually for each of the three replicate fruits, and for each fruit, data points were collected from both cheeks.

Mesocarp position	Day 0	Day 3	Day 6	Day 9	Day 12
Inner	64.0^{a}	61.9^{a}	64.5^{a}	61.9^{a}	60.1^{a}
Middle	61.6^{b}	60.1^{ab}	62.1^{b}	60.3^{a}	58.2^{b}
Outer	58.7^{c}	57.8^{b}	59.7^{c}	58.7^{b}	58.7^{ab}
Treatment					
VHT	61.0^{b}	57.9^{b}	62.5	60.1	58.7
Non-VHT	62.0^{a}	61.9^{a}	61.7	60.4	59.3
Maturity					
More	62.2^{a}	61.6^{a}	63.3^{a}	60.8^{a}	58.3
Optimum	63.2^{a}	61.1^{a}	63.7^{a}	61.3^{a}	58.6
Less	59.1^{b}	57.1^{b}	59.3^{b}	58.8^{b}	60.0

Mean values represented by same letter(s) are not significantly *(P<0.05)* different.

5.3.2 X-ray µCT images

Based on µCT values, mesocarp porosity increased from the inner to the outer mesocarp of 'B74' fruit. Statistical analysis confirmed significant differences (*P<0.05*, Appendix 44) in porosity across different mesocarp regions. Specifically, outer mesocarp exhibited higher porosity, while the middle and inner mesocarp regions had similar porosity (Figure 5.4). X-ray µCT image mapped with the porosity (measured in CT values) of 'B74' mango fruit across various sections of the mesocarp is shown in Figure 5.5.

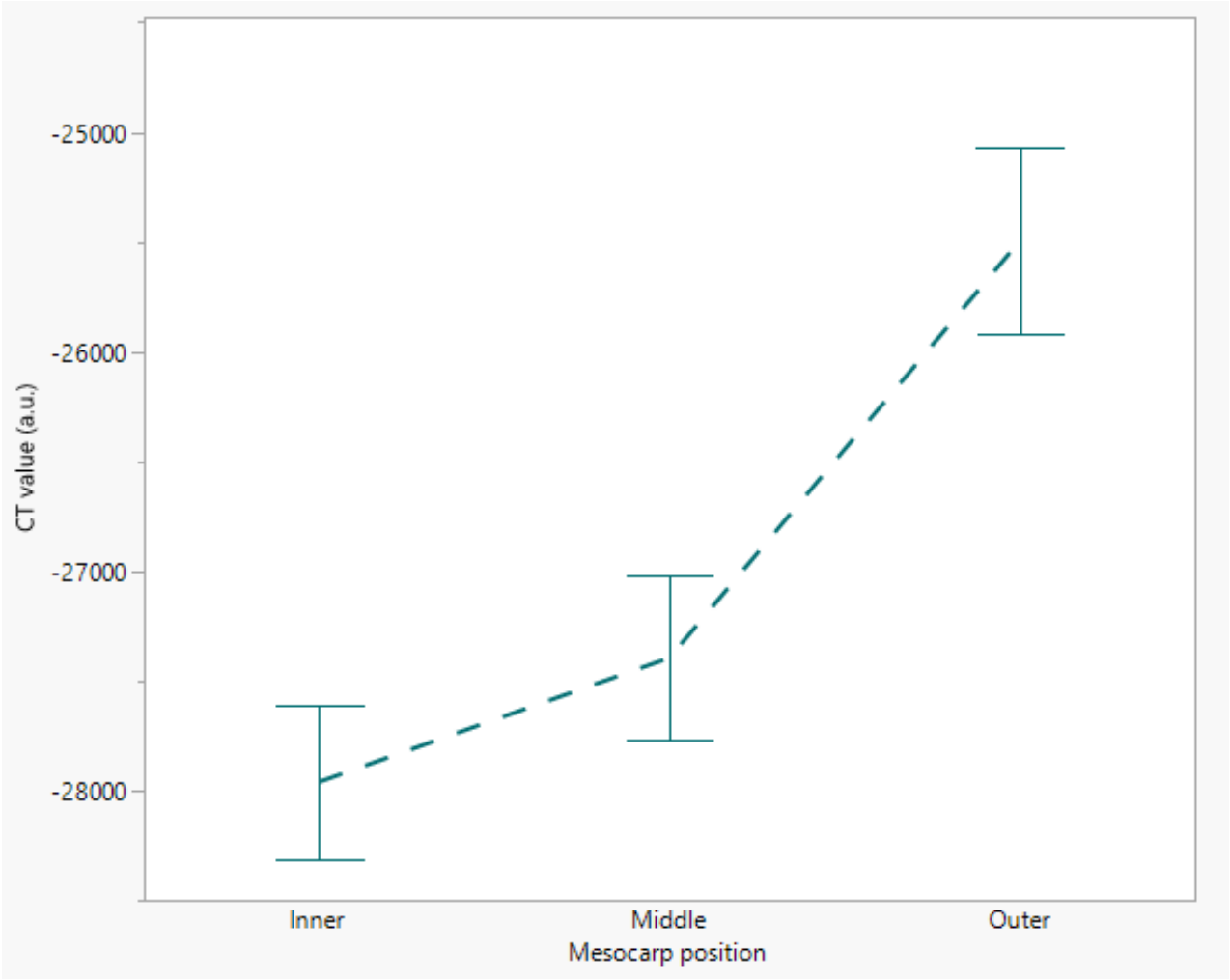


Figure 5.4: Mango fruit mesocarp porosity in CT values of 'B74' mango fruit across the inner, middle, and outer mesocarp of optimum mature fruit. Each data point represents the average porosity value derived from four fruit. Within each mesocarp area, three regions of interest (ROI) were selected per fruit and averaged for the porosity value. Graphic constructed using JMP Pro 16.0.0 (512257). Error bars show standard error of the mean.

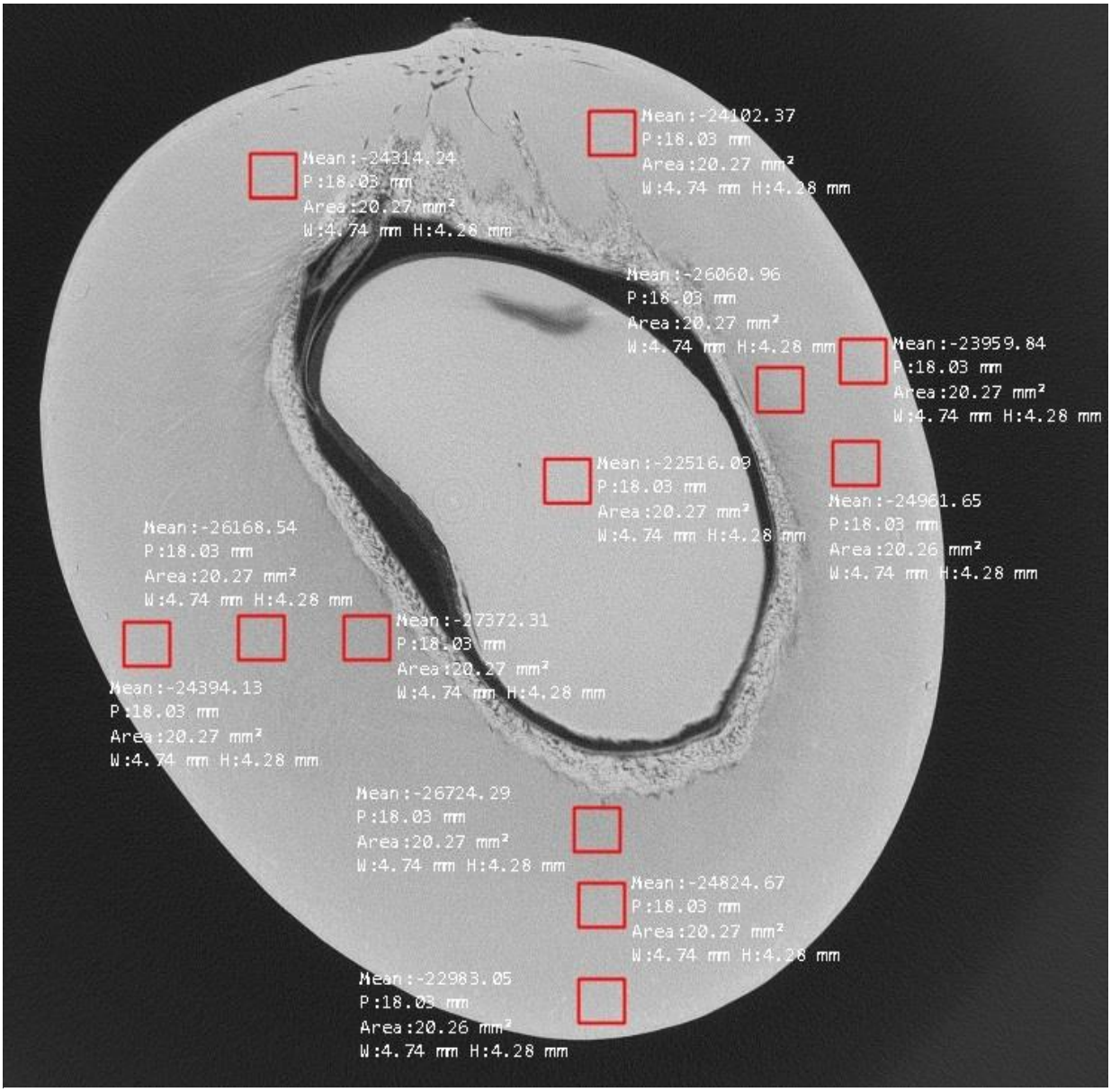


Figure 5.5: X-ray μCT image mapped with CT values in different parts of 'B74' mango fruit mesocarp. Red boxes (with area of 20.27 mm^2) represent the region in which values were calculated along with adjacent CT values as arbitrary units. The MicroDicom DICOM Viewer version 2023.1.1 (Build 1543) 64 bit was used to obtain these image values. The mesocarp displays varying porosity at different positions, with higher CT values indicating higher porosity and vice versa.

5.3.3 Compositional study

5.3.3.1 Fruit firmness

The effect of maturity on the fruit firmness to day 6 was not different *($P > 0.05$)*. However, on days 9 and 12 the maturity effect on firmness was significant *($P < 0.05$;*Table 5.3; Appendix 42). More mature fruit were softer and similar to optimum mature fruit, whereas less mature fruit were

firmer. VHT had a significant *(P < 0.05)* effect on fruit firmness until the end of shelf life. Non-VHT fruit had higher firmness than VHT fruit from day 3 to the end of shelf-life (Table 5.3; Appendix 42). Fruit softening became evident in VHT fruit in day 3. Less mature non-VHT fruit took 6 days to reach the same level of softness as VHT mature and more mature fruit achieved in 3 days (Figure 5.6).

Table 5.3: Effect of maturity (less: <15% DMC, optimum: 15 - 17% DMC, and more mature: >17% DMC), vapour heat treatment (VHT) and interactions on fruit firmness (arbitrary unit) at different stages (from the day after VHT to 12 days after VHT) of 'B74' fruit ripening. Firmness was measured by Fruit firm (TR, Turoni, Forli, Italy). Four measurements were taken from each fruit with two in each check such that measurements represent the whole fruit.

Maturity	Day 3	Day 6	Day 9	Day 12
Less mature	84.3	73.6	61.1[a]	57.1[a]
Optimum mature	81.7	66.5	54.7[ab]	50.5[b]
More mature	77.6	64.7	51.3[b]	48.7[b]
Treatment				
VHT	74.6[b]	61.6[b]	50.7[b]	47.8[b]
Non-VHT	87.7[a]	75.0[a]	60.7[a]	56.4[a]

Mean values represented by same letter(s) are not significantly *(P < 0.05)* different.

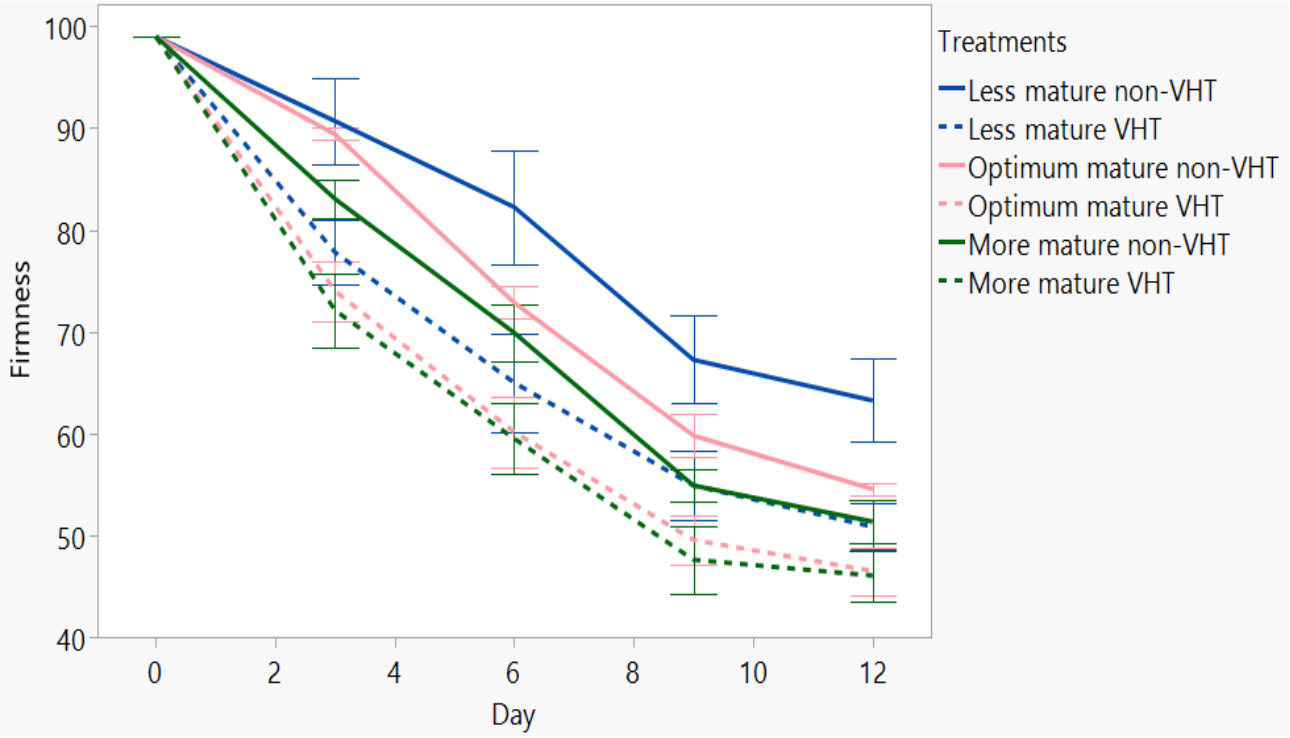


Figure 5.6: Changes in 'B74' mango fruit firmness in compositional study fruit from 1 day after VHT to 12 day after VHT. Each point represents the average of five fruit. Error bars show standard error of the mean. Firmness was measured by Fruit firm (TR, Turoni, Forli, Italy). Four measurements were taken from each fruit with two in each check such that measurements represent the whole fruit.

5.3.3.2 Total soluble solids

Initially, on day 1, there were no differences *(P>0.05)* in TSS among fruit of different maturity. However, on days 3, 6, and 9, differences *(P<0.05)* in TSS were observed among fruit of varying maturity, while no difference *(P>0.05)* was found on day 12 (Table 5.4; Appendix 43). Notably, less mature fruit exhibited lower TSS (P<0.05) than more mature fruit on day 3. The TSS of optimally mature fruit was similar to both less mature and more mature fruit. This trend persisted until day 9. The impact of VHT on fruit TSS did not show significance *(P>0.05)* until day 9. Overall, there was a sharp increase in TSS until day 6, after which TSS remained relatively stable during subsequent ripening. More and optimally mature fruit, excluding optimally mature VHT fruit, exhibited a spike in TSS until day 6. In contrast, less mature fruit and optimally mature VHT fruit displayed a sharp increase in TSS until day 3, with smaller change from day 3 to 6 (Figure 5.7).

Table 5.4: Effect of maturity (less: <15% DMC, optimum: 15 - 17% DMC and more mature: >17% DMC), vapour heat treatment (VHT), and interactions for total soluble solids (TSS, °Brix) at different stages from day after VHT to 12 day after VHT of 'B74' fruit ripening. Fruit were longitudinally sliced on both sides of the 'cheek' near the seed. Juice from each slice was placed onto the prism of a digital pocket refractometer (Atago PAL-1™, Japan), and the values recorded.

Maturity	Day 0	Day 3	Day 6	Day 9	Day 12
Less mature	11.6	14.3b	15.4b	15.4b	15.6
Optimum mature	11.3	14.7ab	16.0ab	15.9ab	16.1
More mature	12.4	15.4a	17.0a	16.5a	16.1
Treatment					
VHT	11.9	14.6	15.7	15.7	15.6
Non-VHT	11.2	15.0	16.5	16.1	16.3

Mean values represented by same letter(s) are not significantly *(P<0.05)* different.

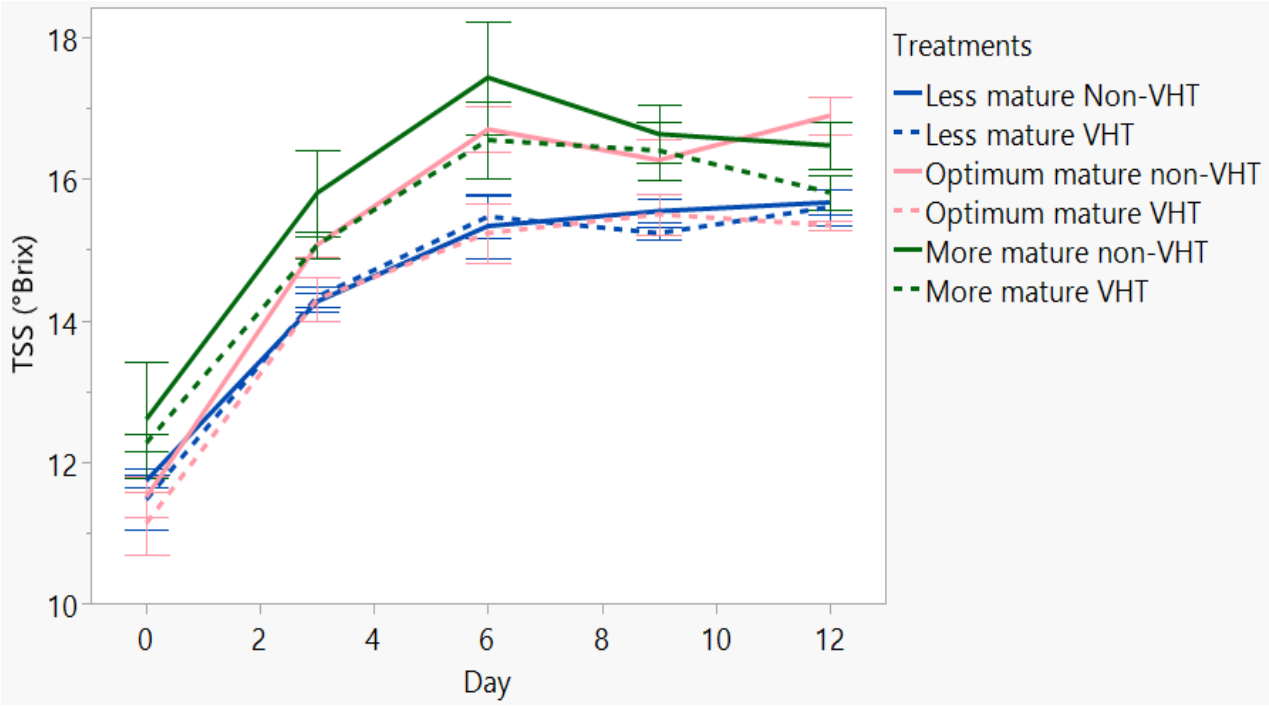
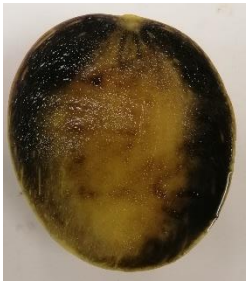


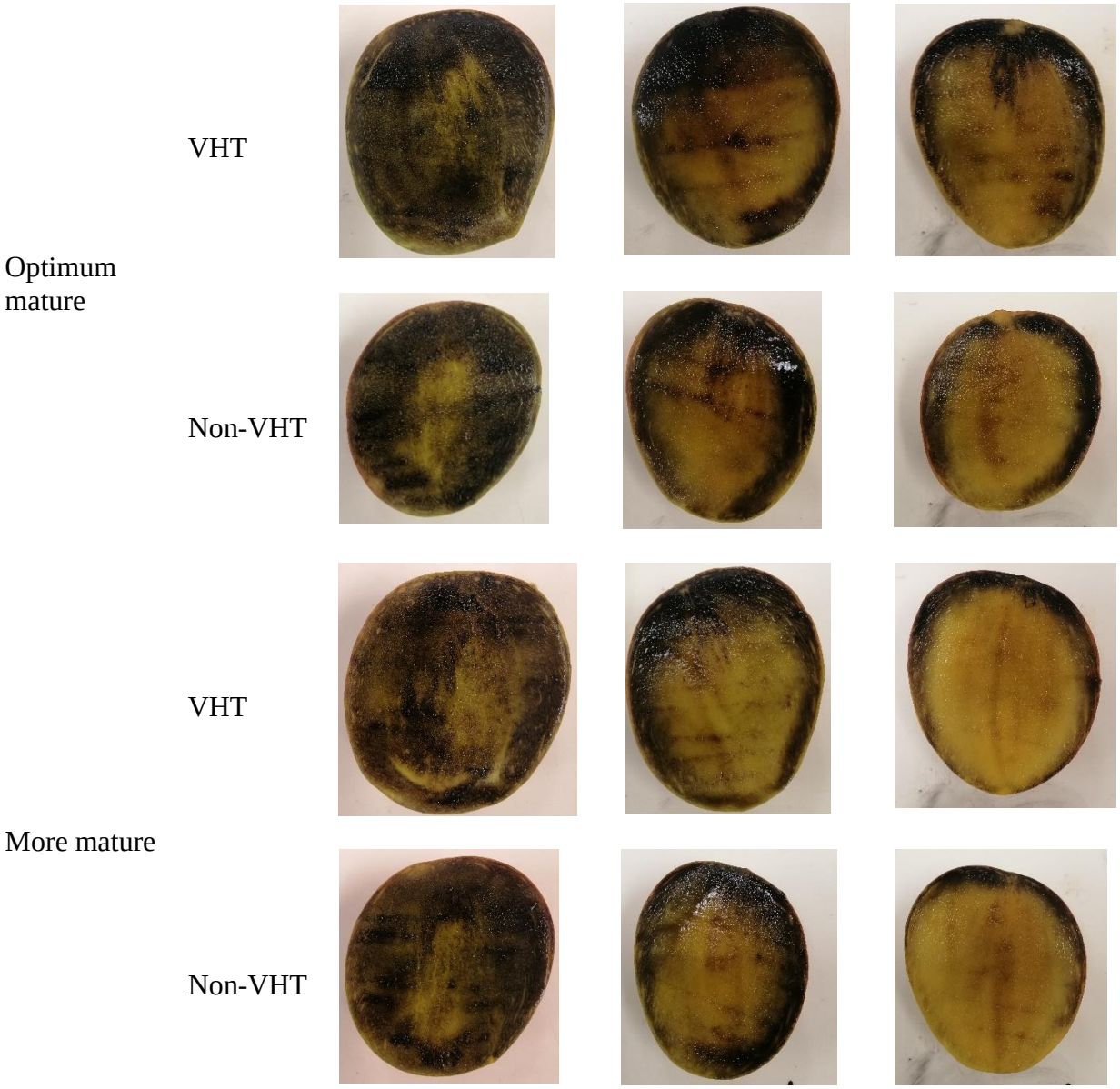
Figure 5.7: Changes in 'B74' mango fruit total soluble solids (TSS) in compositional study fruit from 1 day after VHT (day 0) to 12 days after VHT. Each data point is the average of three fruit. Error bars show standard error of the mean. Fruit were longitudinally sliced on both sides of the 'cheek' near the seed. Juice from each slice was placed onto the prism of a digital pocket refractometer (Atago PAL-1™, Japan), and the values recorded.

5.3.3.3 Starch iodine staining

Starch iodine staining was performed at each imaging event to characterise starch hydrolysis in 'B74' mango fruit at serial ripening stages. On day 0, only minor starch hydrolysis had occurred in the centremost region of the fruit flesh as evidenced by dark stained starch across most of the mesocarp (Table 5.5). By day 3, noticeable increase in starch hydrolysis was observed compared to day 0. This increase was characterised by a shift of the dark area towards the fruit skin, exposing the underlying yellow fruit flesh (Table 5.5). Progressing to day 6, the starch stain further migrated closer to the fruit skin, predominantly concentrated in the stem end of the fruit (Table 5.5). In terms of different mature fruit, on day 3 the band of starch stain was relatively less in more mature fruit indicating significant starch hydrolysis evident from day 3 onwards. By day 6, almost all the starch was hydrolysed in more mature fruit with starch stain being observed only in the skin while in optimum and less mature fruit starch stain was observed in the outer mesocarp and in the stem end of the fruit (Table 5.5).

Table 5.5: Images of longitudinal sections of 'B74' mango fruit stained for starch with Lugol's solutions for three-imaging events i.e., days 0, 3, and 6 post VHT. Black regions are starch stained with iodine. Yellow flesh reflects breakdown of the starch to sugar in the flesh.

	Day 0	Day 3	Day 6
VHT			
Less mature Non-VHT			



5.4 Discussion

Mango fruit are typically harvested at green mature stage and subsequently ripen. The duration between harvesting and consumption can span several weeks. During maturation and ripening, physiochemical changes occur in the different parts of the mesocarp. These changes are characterised by non-uniform water activity observed throughout the mesocarp (Joyce *et al.,* 2002). An understanding of these changes during ripening is important for interpretation of MRI and µCT imaging.

MRI and X-ray µCT are suitable methods for visualising physiochemical changes *in vivo* during ripening (Suchanek *et al.,* 2017; Zhou *et al.,* 2019). Such imaging techniques provides image contrast by exploiting internally generated interface between air and liquid-filled pores (Defraeye *et al.,* 2013). The magnitude of these gradients depends on factors such as intercellular spaces (porosity),

distribution of pore sizes, and pore shape (Defraeye *et al.,* 2013). In this study, 'B74' mango fruit were imaged by MRI for 12 days after VHT at 3 days intervals and X-ray µCT after VHT, to study the temporal and spatial physiochemical gradients in the fruit mesocarp.

Signal intensity for T2 (Table 5.1, Appendix 48) and PD (Table 5.2, Appendix 49) values, was higher *(P<0.05)* in the inner mesocarp, and gradually decreased towards the outer mesocarp. This gradient was seen in MR images as a transition from lighter to darker regions evident on days 3 and 6 (Figure 5.8). Joyce *et al.,* (2002) working with 'Kensington Pride' mango fruit also reported that signal intensity was highest near the seed and lowest near the skin. This difference in signal intensity between parts of the mesocarp can be attributed to presence of more free or mobile water molecules in the inner mesocarp (Schotsmans *et al.,* 2004; Verboven *et al.,* 2008; Mendoza *et al.,* 2010). Chen *et al.* (1989) reported that regions with higher free water exhibited higher intensity in T2 and PD images in different fruit and vegetables. This is consistent with the lower DMC in inner mesocarp of mango fruit compared to outer mesocarp (Joyce *et al.,* 2002). Higher water mobility in inner tissue may be due to initiated ripening of 'B74' mango fruit starting from the inner mesocarp, as was evident from starch staining (Table 5.5).

Higher T2 and PD values can reflect lower porosity, higher moisture content, and larger pore size of the inner tissue (Schotsmans *et al.,* 2004; Verboven *et al.,* 2008; Mendoza *et al.,* 2010). X-ray µCT imaging also suggested lower porosity in the inner mesocarp, reflecting a putative porosity gradient from lower to higher from inner to outer mesocarp (Figures 5.6 and 5.7). Cantre *et al.* (2020) and Cantre *et al.* (2014) found that the outer mesocarp of 'Carabao' mango fruit exhibited higher porosity, with more interconnected pores, compared to the inner mesocarp. Over time, porosity of both the inner and outer mesocarp decreased and reached similar levels by the end of storage. This was evident in that there were no differences in T2 values *(P>0.05)* in different parts of the mesocarp on day 12 (Table 5.1).

The change in mesocarp porosity during ripening can impact gas exchange and fruit respiration (Lammertyn *et al.,* 2003a; Lammertyn *et al.,* 2003b). This may explain why inner mesocarp is relatively more susceptible to the browning disorder. More mature fruit exhibited higher T2 (Table 5.1), and PD (Table 5.2) values *(P<0.05)* compared to less mature fruit. Higher signal intensity in more mature fruit may be attributed to increased free water as typically associated with faster ripening (Chen *et al.,* 1989). Composition assessments likewise revealed that the more mature fruit ripened faster as evidenced by starch staining (Table 5.5), fruit firmness (Figure 5.6 and Table 5.3), and TSS (Figure 5.7 and Table 5.4) assessments. These findings suggest that fruit of mixed maturity within batches should be strictly avoided. That is, because more mature fruit become more

susceptible to deterioration by the time they reach marketers and consumers. This was evidenced as greater changes seen in more mature fruit by MR images (Figure 5.8).

Initiation of ripening from the inner mesocarp is associated with deterioration of cell membranes, electrolytes leakage and cell sap displacement of air space (Cantre *et al.,* 2020) apparent as an increase in T2. This change in proton mobility was also associated with starch breakdown, reduction in acidity, and other ripening-related processes that evidently commence near the seed (Joyce *et al.,* 2002). The T2 of sugar solutions decreases as sugar content increases (Musse *et al.,* 2009). Similarly, T2 relaxation times in mango mesocarp decrease during ripening. T2 and PD values in plant tissues are sensitive to water content, sugar concentration, cell dimensions, and membrane permeability (Musse *et al.,* 2009).

Apparent movement of available free water into adjacent regions, such as towards the seed and outer mesocarp, resulted in a decrease in the T2 value. This phenomenon is suggested by MR images where bright regions near the seed on day 3 or 6 were no longer observed on day 9, suggesting the relocation or redistribution of free water to other regions (Figure 5.8). Defraeye *et al.,* (2013) reported that initially PD, diffusion coefficient, and T2 value of the tissue affected by internal browning or bruising were higher than those of the healthy tissue due to the free water from the destruction of cellular integrity. However, sometime after injury T2 and PD values was decreased in the injured regions (McCarthy *et al.,* 1995) because of movement of free water to the adjacent region leaving the injured area dry.

At end of shelf-life, cavities were observed in all over-mature for VHT and non-VHT and optimum mature for VHT only fruit in the inner mesocarp (green circles, Figure 5.8). However, cavities at end of shelf life in less mature fruit were not observed other than when induced by VHT at earlier stage ripening. The presence of cavities near the seed at end of shelf-life lends support to the proposition that changes in cellular structures during ripening. Such change was apparently greater in inner mesocarp and in more mature fruit. Lammertyn *et al.* (2003a) also reported partial destruction of cellular integrity during ripening prior to formation of large cavities. Such processes evidently facilitate migration of water to different regions of the fruit, including towards the seed and / or the outer mesocarp, prospectively contributing to formation of cavities. Notably, flesh cavity near the seed was observed exclusively in less mature VHT fruit, as marked with a red circle in Figure 5.8. The size of the cavity increased from day 0 to day 3 and beyond supporting the proposition of movement of water from the region of injury into adjacent regions. VHT-induced cavity in less

mature fruit reported in Chapters 3 and 4 was termed ‘flesh cavity with white patches’ (FCWP). Its relations with fruit maturity is also discussed in detail there.

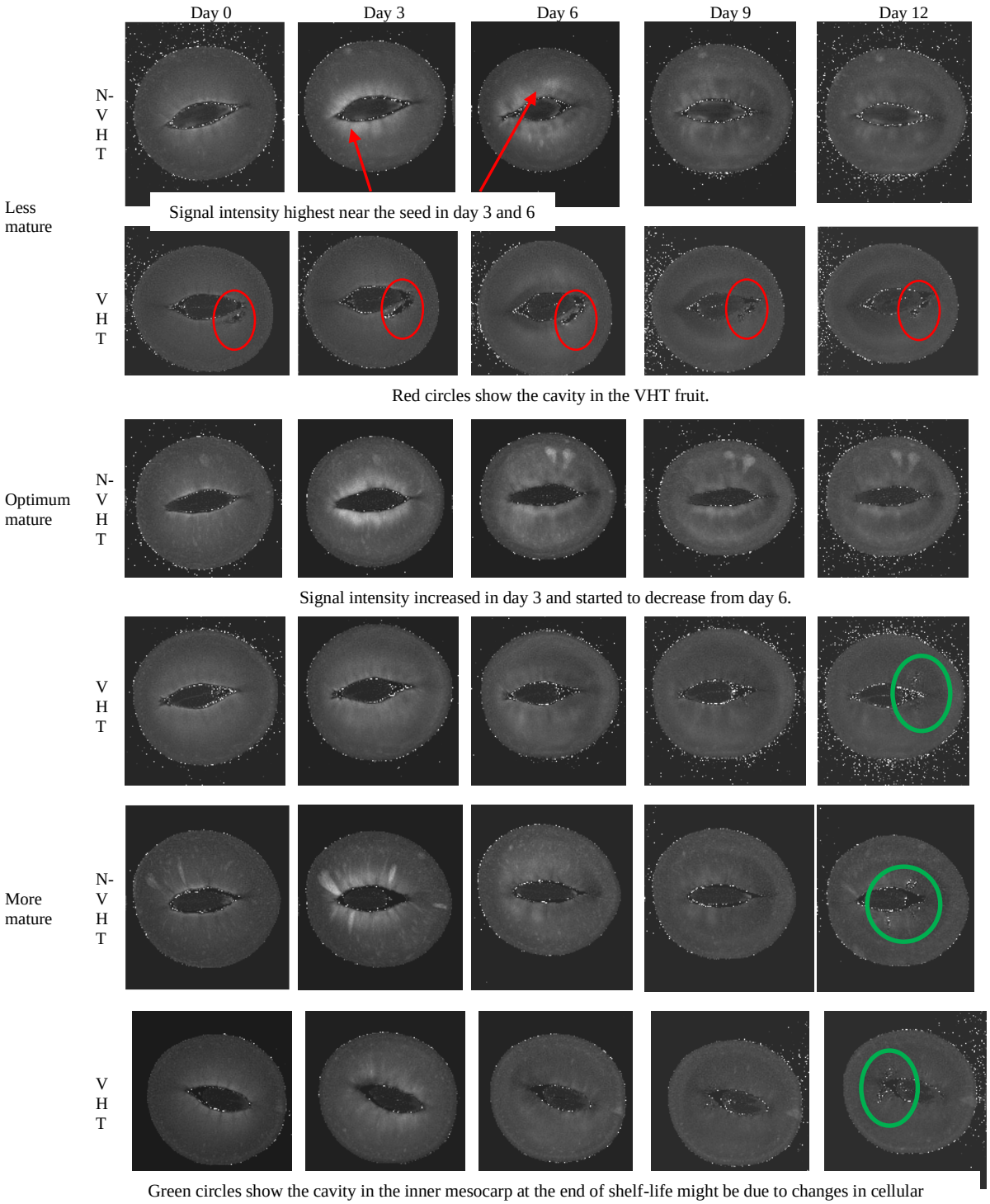


Figure 5.8: T2 images of ‘B74’ mango fruit with different maturities (less mature: <15% DMC, optimum mature: 15-17% DMC, and more mature: >17% DMC) at different stages of ripening (day after VHT to end of shelf-life i.e., 12 days) in 3 days interval with and without VHT.

5.5 Conclusions

The ripening process of mango fruit is characterised by a variety of physiochemical changes that significantly influence fruit composition and structure. Gaining a comprehensive understanding of these changes is essential for effective postharvest handling and quality control. While the physiochemical, biochemical, and compositional changes during mango fruit ripening have been extensively studied, there is a lack of detailed characterisation of the spatial and temporal changes in physiochemical gradients within the mango fruit mesocarp.

Spatial and temporal changes in 'B74' mango fruit during the ripening process were characterised. They explored physiochemical gradients within ‘B74’ mango mesocarp. The most pronounced changes were seen in the inner mesocarp, gradually extending outwards. Concomitantly, porosity of the inner mesocarp was lower compared to outer mesocarp.

Such changes reflecting dynamic cell structure and function have implications for gas exchange and overall fruit physiology. Deterioration in cellular integrity, particularly in the inner mesocarp, leakage of electrolytes and their relocation to adjacent regions evidently contribute to the of cavity formation within the fruit. By establishing a link between physiochemical properties and fruit quality, better understanding for postharvest management and overall quality ’B74’ mango, along with other cultivars, is enabled. This contributes to optimization of postharvest treatment and handling conditions.

Further studies are warranted to validate these findings with high-resolution images and increased samples sizes. Herein, imaging was conducted at 3-day intervals. Shorter intervals for imaging may inform deeper understanding of the spatial and temporal physiochemical changes associated with ripening. In this study, X-ray µCT imaging was performed at commencement of the experiment. Considering proof of principle herein, it should be applied temporally in concert with MRI. Supplementing such research with complementary ‘high resolution’ biochemistry is an attendant opportunity.

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Chapter 6 Internal disorders prediction of ‘B74’ mango fruit using in-line near infrared spectroscopy

6.1 Introduction

Post-harvest issues in ‘B74’ mangoes include flesh browning (FB) and flesh cavity with white patches (FCWP) (Chapter 1, Figure 1.5). These internal disorders (IDs) result in fruit rejection and economic loss (Holmes 2019a, 2019b). Thus, mangoes with IDs are poor quality and a commercial concern.

Early in the supply chain, external signs of IDs are typically absent. Additionally, some disorders may only become apparent at the ripe stage (Gabriëls *et al.*, 2020). Ideally, disorders should be predicted and identified early in the supply chain. Selecting for robust high-quality fruit requires reliable, affordable, accurate, and non-destructive technology (Gabriëls *et al.*, 2020; Huang *et al.*, 2020; Mogollón *et al.*, 2020).

A range of non-destructive techniques, including visible and near-infrared spectroscopy (Vis-NIRS), magnetic resonance imaging (MRI), X-ray, hyperspectral and multi-spectral imaging, and acoustics have been investigated and applied for fruit and vegetables (Jha *et al.*, 2010). Among them, Vis-NIRS has gained acceptance for rapid, accurate, and cost-effective quality detection and prediction (Nicolai *et al.*, 2007; Gabriëls *et al.*, 2020). Various applications relating to mango fruit include prediction of dry matter content (represented as a percentage of dry weight to fresh weight; DMC), total soluble solids (TSS), firmness, and flesh colour (Subedi *et al.*, 2007; Rungpichayapichet *et al.*, 2016; Nordey *et al.*, 2017). In contrast, its potential to predict IDs in mango is relatively unexplored.

Studies on detection / prediction of IDs by Vis-NIR include brown heart in pears (Fu *et al.*, 2007) and apples (Clark *et al.*, 2003; McGlone *et al.*, 2005), brown core in Chinese pear (Han *et al.*, 2006), FB in apples (Torres *et al.*, 2014; Khatiwada *et al.*, 2016; Huang *et al.*, 2020; Mogollon *et al.*, 2020), bitter pit in apples (Nicolaï *et al.*, 2006; Kafle *et al.*, 2016; Jarolmasjed *et al.*, 2017), and translucent flesh disorder in mangosteen (Teerachaichayut *et al.*, 2007). Collectively, they suggested that Vis-NIRS may be used for prediction of IDs in mango.

Proof of principle research on in-line Vis-NIRS applications for predicting internal quality is expanding, but most studies are conducted under static conditions (Sharma *et al.*, 2020). The spectroscopic and chemometric theory of operation for fresh produce analysis is well-established, and dedicated instrumentation for the postharvest sector is available (Walsh *et al.*, 2020b). Commercial

systems can assess fruit on a moving belt at 1 m/s (Walsh *et al.*, 2020b). However, application and adoption of spectroscopic technologies for predicting IDs in commercial postharvest practices is relatively limited. Therefore, this study investigated the efficacy of in-line Vis-NIR towards predicting IDs in 'B74' mango fruit sorted at hard green mature stage.

6.2 Materials and methods

6.2.1 Fruit source

Fruit was sourced and handled as per Chapter 3, Section 3.2.2.1. Four hundred fruit from each of Northern Territory (NT) and North Queensland (NQ) regions were randomly sampled in the VHT facility, Rocklea, Brisbane. They were then transported (~75 km) to the postharvest laboratory at Gatton Campus in an air-conditioned car. The following day, the fruit were taken to Gayndah (~325 km) in an air-conditioned car for NIR spectra acquisition to predict IDs.

6.2.2 Spectra acquisition

NIR spectra were collected on Inspectra^2 unit (TOMRA Food, Leuven, Belgium) fitted with the Zeiss monolithic miniature 1 spectrometer (Carl Zeiss Spectroscopy, Jena, Germany) with Hamamatsu sensor and a tungsten halogen lamp for illumination.

Reflectance spectra ranging from 310 - 1100 nm of intact mangoes was collected from opposite sides of the widest section of each cheek at the equatorial region. Fruit was hard green mature at spectra acquisition. A total of 1600 spectra were collected from 800 mangoes in total. Each fruit was manually positioned at the centred of the holder, which moved at a speed of 0.5 m/s. The integration time was 4 ms, and ~47 spectra per fruit, depending on its size, were collected. After data cleaning and pre-processing, ~28 spectra per fruit were utilised. Thereafter, fruit were transported to the postharvest laboratory at The University of Queensland, Gatton Campus (~325 km). The following day, they were transferred to Rocklea, Brisbane (~75 km) in an air-conditioned car for VHT.

6.2.3 VHT, shelf-life, and IDs assessment

These practices were performed as outlined in Chapter 3, Sections 3.2.2.3, 3.2.2.4, and 3.2.2.5.

6.2.4 DMC and TSS reference value measurement

At the time of IDs assessment, oven-DMC was also assessed (Chapter 4, Section 4.2.1.2) along with TSS (Chapter 5, Section 5.2.3.2). These parameters were assessed on both sides of fruit. However, four fruit were lost due to disease and so 796 fruit in all were used for reference values.

6.2.5 Data analysis

Data were pre-processed by eroding edge spectra to avoid steep surface angle and saturated spectra were removed. They were standardised by subtracting a dark reference and division by the difference between white and dark reference values. These references were in the instrument's hardware. Zeiss quadratic correction coefficients was applied for wavelength correction. Zeiss quadratic coefficients was for the fit (calibration) between the 256 pixels of the detector diode array and the wavelengths of light received after grating dispersion. Pixels have uniform wavelength spacing but they vary. Therefore, spectra were resampled onto a standard wavelength template to interpolate the data. Any wavelength <467 nm or >1053 nm was removed, and 200 wavelengths overall were used. Wavelengths <467 nm and >1053 nm have low signal to noise ratio, due to lack of light, and / or lack of spectrometer sensitivity. Spectra normalisation (The sum of the squares of spectra values sums to 1) was adopted using L2 norm to render results independent of fruit size, lamp brightness, spectrometer sensitivity, and distance of fruit surface from lamp / spectrometer. L2 normalization scales the data in such a way that the Euclidean norm (the length or magnitude of the vector) of each data point is equal to 1. This helps to eliminate the influence of the absolute size of the data, making it suitable for comparisons. All spectra within one fruit averaged to create single spectrum (R. Valkenburg, Per. Comm.).

A 4-fold cross validation was used. Thereby, the data were split into four quarters, and the four possible permutations of ¾ training and ¼ validation were created, and the results combined. Partial least square (PLS) regression with nonlinear iterative partial least square (NIPALS) was performed on the whole original spectra to develop the NIR model with the number of principal components / latent variables set to 25. PLS-discriminate analysis (DA) was used for IDs classification. The classification model was made using following metrics: accuracy (viz., proportion of fruit correctly classified into each severity class), sensitivity (viz., proportion of disordered fruit correctly classified), and specificity (viz., proportion of healthy fruit correctly classified) (Gabriëls *et al.,* 2020). The classification model was made in two ways: based on severity classes (0 - 3 severity rating scale), and binary classification (healthy and disordered).

6.3 Results

6.3.1 Distribution of DMC, TSS and IDs

Nir-DMC ranged from 11.1% - 20.8% (average: 15.6%) (Table 6.1, Figure 6.1). TSS ranged from 10.1 - 19.6 °Brix (average: 14.2 °Brix) (Table 6.1, Figure 6.1). FCWP and FB severity in the fruit lot ranged from 0 - 3 (Figure 6.2). Among them, 56% fruit were affected by FCWP: 32%, 19%,

and 5% had low, moderate, and high severity, respectively. Similarly, 11% of the fruit were affected by FB: 8%, 2%, and 1% had low, moderate, and high severity, respectively (Figure 6.2).

Table 6.1: Descriptive statistics of DMC, TSS for ‘B74’ mango fruit used for the internal disorders (IDs) prediction by in-line near infra-red (NIR) spectroscopy. Oven-DMC and TSS were assessed at the end of shelf-life by oven dry and digital refractometer, respectively.

Internal quality	n	mean	max	min	SD
DMC	1592	15.6	20.8	11.1	1.6
TSS (°Brix)	1592	14.2	19.6	10.1	1.6

DMC: dry matter content; TSS: Total soluble solids; FCWP: flesh cavity with white patches; FB: flesh browning; n = number of data (total fruit number × readings from each fruit); SD: standard deviation; min: minimum; max: Maximum.

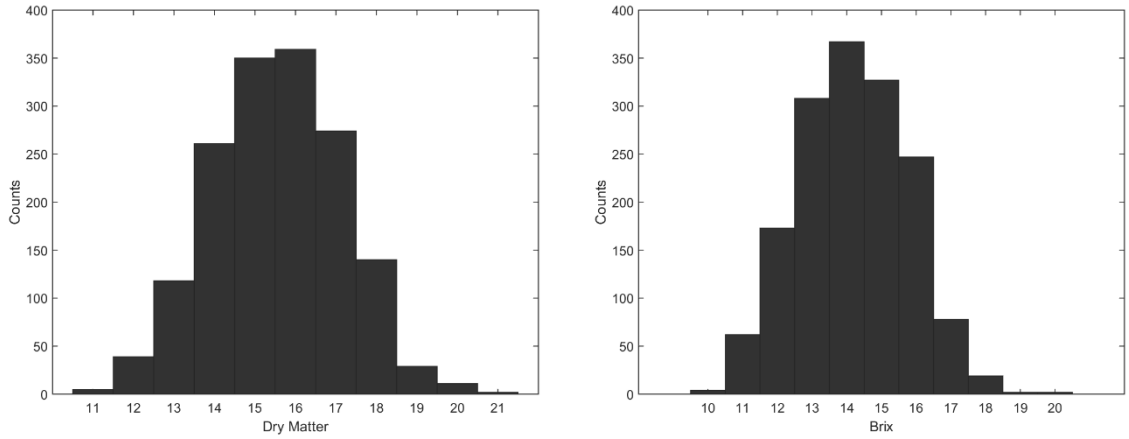


Figure 6.1: Frequency distributions of dry matter content (DMC; left) and total soluble solids (TSS; right) of ‘B74’ mango fruit used for the internal disorders (IDs) prediction by in-line near infra-red (NIR) spectroscopy. DMC and TSS were measured at the end of shelf-life by oven dry method and by digital refractometer, respectively.

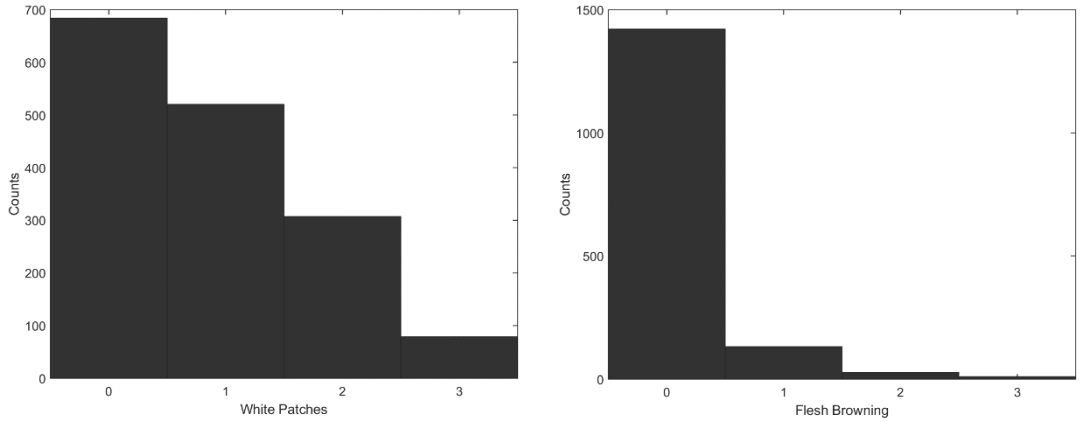


Figure 6.2: Frequency distributions for flesh cavity with white patches (FCWP: left) and flesh browning (FB: right) of 'B74' mango fruit. Internal disorders (IDs) were rated on a severity rating scale of 0 - 3; 0: healthy and 3: severe. IDs were visually assessed at eating soft stage by slicing both sides of fruit closer to the seed.

6.3.2 Relationship between measured DMC and TSS

Oven-DMC was correlated (r^2 = 0.8, *P*<0.0001) with TSS measured at the ripe stage (Figure 6.3). Fruit with higher oven-DMC exhibited higher TSS when they reached the ripe stage. Fruit with >15% DMC had minimum of 14 °Brix TSS, which is the market specification of Australia (Mango Matters, 2017).

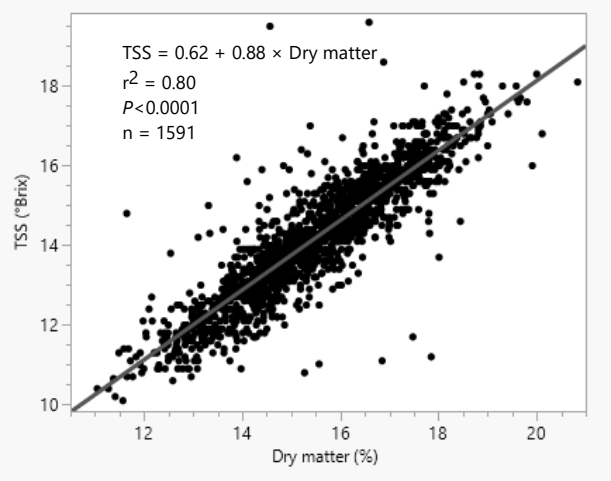


Figure 6.3: Correlation between dry matter content (DMC) and total soluble solids (TSS) assessed at eating soft stage. DMC and TSS were measured at the end of shelf-life by oven dry and digital refractometer, respectively.

6.3.3 Relationship between measured oven-DMC and internal disorders

Consistent with findings reported in Chapters 3 and 4, maturity was *(P<0.05)* associated with FCWP and FB incidence. Less mature fruit had both lower FB incidence and severity (Table 6.2). In contrast, more mature fruit had lower FCWP incidence *(P<0.05)* compared to optimally mature fruit,

but like less mature fruit. Although the incidence of FCWP was similar between less and more mature fruit, its severity differed *(P<0.05)* between the two maturity classes (Table 6.2).

Table 6.2: Flesh cavity with white patches (FCWP) and flesh browning (FB) incidence and severity in fruit of different maturity, i.e., less mature (<15% DMC, n = 630), optimum mature (15 - 17% DMC, n = 676), and more mature (>17% DMC, n = 286) fruit. DMC was assessed by oven drying method.

Fruit maturity	FCWP		FB	
	Incidence %	Severity	Incidence %	Severity
Less mature (<15% DMC)	55.4^{ab}	1.0^{a}	5.6^{b}	0.1^{b}
Optimum mature (15 - 17% DMC)	62.1^{a}	0.9^{ab}	11.4^{ab}	0.2^{ab}
More mature (>17% DMC)	49.8^{b}	0.6^{b}	17.9^{a}	0.3^{a}

Mean values within a column represented by same letter(s) are not significantly different.

6.3.4 DMC and TSS prediction by in-line NIR

Table 6.3 and Figure 6.4 summarise the in-line NIR prediction models statistics for DMC and TSS. In-line NIR prediction for both parameters showed a strong correlation between measured and predicted values with coefficient of determination (r^2) of 0.8. The root mean square error of prediction (RMSEP) was 0.7, indicating low prediction error. The models have a bias of 0.0, a slope of 1.0, and RPD of 2.4, demonstrating their accuracy and reliability. Overall, the results indicate promising predictive capabilities for DMC and TSS.

Table 6.3: In-line near infra-red (NIR) prediction statistics for DMC and TSS in 'B74' mango fruit. NIR spectra were collected at hard green mature fruit and reference values were assessed at eating ripe stage. DMC and TSS were assessed by oven dry and digital refractometer, respectively.

Internal quality	n	Validation				
		r^2	RMSEP	Bias	Slope	RPD
DMC	1592	0.8	0.7	0.0	1.0	2.4
TSS (°Brix)	1592	0.8	0.7	0.0	1.0	2.4

DMC: dry matter content; TSS: Total soluble solids; n = number of data (total fruit number × readings from each fruit); r^2 = coefficient of determination, RMSEP: root mean square of prediction, RPD: residual prediction deviation.

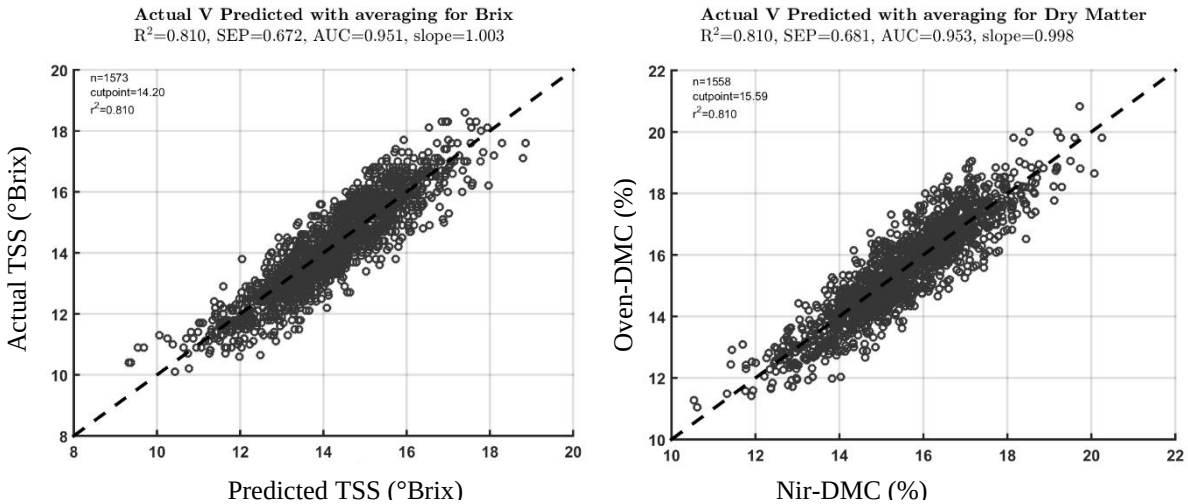


Figure 6.4: Relationship between in-line near infra-red (NIR) predicted and measured total soluble solids (TSS; left) and dry matter content (DMC; right) of 'B74' mango fruit. NIR spectra were collected at mature hard green stage for the prediction and reference values was assessed at eating ripe stage.

6.3.5 IDs prediction by in-line NIR

The study revealed that in-line NIR had low accuracy (47.4%), sensitivity (54.9%), and specificity (43.4%) for classifying FCWP disorder based on severity rating scale (Table 6.4). However, the binary classification model demonstrated promising outcomes for FCWP, with an accuracy of 72.5%, sensitivity of 78.3%, and specificity of 64.8% (Table 6.5).

For FB classification using the severity rating scale, the accuracy and specificity were 90.5% and 98.7%, respectively (Table 6.4). In the binary classification model, the accuracy and specificity were 91.1% and 99.9%, respectively (Table 6.5). While the overall accuracy was high for FB classification, the sensitivity was 3.7% and 0% for severity based and binary classification, respectively indicating limitations in detecting the disorders. Confusion matrix for identifying IDs FCWP and FB by in-line NIR based on severity rating scale is presented in Appendix 46.

Table 6.4: Internal disorders (IDs) classification model (viz., accuracy, sensitivity, and specificity) based on severity ratings 0 - 3 (0: healthy and 3: severe disorder) by in-line near infra-red (NIR). NIR spectra were collected at mature hard green stage for IDs prediction.

Classification model	Validation data set		
	Accuracy	Sensitivity	Specificity
Flesh browning*	90.5%	3.7%	98.7%
Flesh cavity with white patches	47.4%	54.9%	43.4%

*Flesh Browning data mostly comprised healthy fruit, so the overall accuracy with no classification of IDs is very high.

Table 6.5: Internal disorders (IDs) binary (healthy and disorder) classification model (viz., accuracy, sensitivity, and specificity) by in-line near infra-red (NIR). NIR spectra were collected at mature hard green stage for the IDs prediction.

Classification model	Validation data set		
	Accuracy	Sensitivity	Specificity
Flesh browning*	91.1%	0%	99.9%
Flesh cavity with white patches	72.5%	78.3%	64.8%

*Flesh Browning data mostly comprised healthy fruit, so the overall accuracy with no classification of IDs is very high.

6.4 Discussion

The ‘B74’ mango fruit exhibited significant variation in DMC and TSS within the fruit lot (Table 6.1, Figure 6.1). Such variation is common phenomenon in mango supply chains (Ainsworth, 2016). Variation in fruit maturity at harvest arises from the non-uniform maturation within and between trees, including because, for example, flowering and fruit set take place over several weeks (Lakshminarayana, 1973). Fruit of different maturities within a single batch leads to heterogeneous quality and can disappoint consumers.

DMC at harvest is hydrolysed into TSS at ripe stage and accordingly measured DMC and TSS exhibit strong correlation (r^2 = 0.8, *P<0.0001*) (Figure 6.2). Ripe eating quality of mango was determined based on TSS (Saranwong et al. 2004; Subedi et al. 2007). Therefore, DMC at harvest largely determine ripe fruit eating quality of mangoes and serves as an important harvest index (Saranwong *et al.*, 2004; Zhang *et al.*, 2019). ‘B74’ mango fruit with <15% DMC at harvest are considered physiologically immature (Mango Matters, 2017). Fruit with ≥15% DMC generally reach a minimum TSS of 14 °Brix when ripe (Figure 6.2), which aligns with Australian market specifications (Mango Matters, 2017).

FCWP and FB were two major IDs observed in this study (Section 6.3.1). Prediction accuracy for FCWP based on the severity rating scale was only 47.4% (Table 6.3). Moreover, FB prediction was not promising. The relatively low prediction accuracy suggests that more fruit in each severity class is required for model training. Utilizing binary prediction (i.e., healthy, and disordered) instead of a severity class-based prediction, improved FCWP prediction accuracy (72.5%). Hence, modifying the rating scale for disorders may enhance prediction accuracy.

Walsh *et al.* (2020a) affirmed that NIR relies on assessing attributes existing at the time of measurement. Since both IDs were not present in the fruit at the time of spectra acquisition (viz., FCWP appeared after VHT and FB appeared at the ripe stage), NIR may be limited with respect to accurately differentiating ultimately disordered from healthy fruit. Chemometric differences in the fruit that are susceptible to FCWP and FB at the hard green mature stage may not exist or may be negligible. Mogollón *et al.* (2020) observed that disorders occurring during later stages of ripening pose challenges for prediction using at-harvest measurements. They achieved 65% accuracy in predictions based on spectra collected at harvest, while spectra collected after storage yielded 76% accuracy. The improvement in accuracy was possibly due to interim development of disorder symptoms presenting detectable physical and / or chemical changes for NIR analysis. Gabriëls *et al.* (2020) found distinct NIR signatures between the healthy and internal browned mangoes when the disorder was present during assessment, indicating different physiochemical properties in mangoes affected by internal browning compared to healthy ones. Several other studies also successfully detected the internal defect in fruit when the disorders were present, including (Clark *et al.*, 2003; Upchurch *et al.*, 1997; McGlone *et al.*, 2005; Vanoli *et al.*, 2010; Vanoli *et al.*, 2014; Huang *et al.*, 2020; Mogollon *et al.*, 2020). Thus, spectra acquisition after VHT for FCWP and at ripe stage for FB may enable NIR to classify healthy and disorder fruit with more accuracy.

Another possible reason for lower accuracy of disorder prediction may be insufficient penetration (*ca.*, 10 mm) of NIR for assessment of beneath the exocarp (Lammertyn *et al.*, 2000; Marques *et al.*, 2016; Gabriëls *et al.*, 2020). IDs typically manifest from 3-5 cm beneath the epidermis. Moreover, there may not be or be so much chemometric or physiochemical differences in flesh above the inner and middle mesocarp. Nevertheless, Gabriëls *et al.* (2020) observed that NIR captured secondary effects associated with changes potentially reflecting internal browning in the outer mango mesocarp. Walsh *et al.* (2020a) also reported NIR predicting by correlation the expression of a future attribute. For example, DMC measured at harvest is linked to final eating quality (TSS) (Saranwong *et al.*, 2004; Zhang *et al.*, 2019; Subedi *et al.*, 2007).

In the present study, IDs were linked to fruit maturity (DMC) (Table 6.2). Consistent with previous findings (Chapters 3 and 4), less mature fruit displayed greater sensitivity to FCWP and more mature fruit to FB (Table 6.2). Optimally mature fruit exhibited higher tolerance to various physiological disorders (Arpaia, 1994), viz., watery pulp breakdown (Bally, 2006), stem end cavity (Hofman and Whiley, 2010), FCWP (Khanal *et al.*, 2022), and jelly seed (Yahia, 1998; Yahia, 2005; Hofman and Whiley, 2010) in mango fruit. Thus, prediction of fruit maturity (DMC) using in-line NIR in correlating to oven-DMC was reasonably accurate (r^2 = 0.8, SEP = 0.7) (Table 6.3). Thereby, in-line NIR sorting fruit based on maturity to grade out and selectively direct relatively more tolerant fruit into mandatory phytosanitary treatment, like VHT, export supply chains is a demonstrably viable strategy.

Successful implementation of NIR in the fruit industry relies on its robustness as a tool (Anderson *et al.*, 2020). Based on the findings reported herein, enhancing prediction accuracy might be effected improving the model using a more diverse range of data and / or adopting a different regression model. Gabriëls *et al.* (2020) reported that PLSR exhibited lower predictability (r^2 = 0.5) than artificial neural network (ANN; r^2 = 0.7).

Ideally, NIR should be capable of sorting fruit with 80% accuracy to be commercially reliable (Guthrie *et al.*, 1998). Saeys *et al.* (2005) and Davey *et al.* (2009) stressed the significance of validating prediction models through $\mathrm{RPD} = \frac{\mathrm{SD}}{\mathrm{RMSEP}}$values. RPD values between 2.0 - 2.5 are suitable for quantitative prediction (2.4 in this study for DMC and TSS (Table 6.3), and values above 2.5 are considered excellent prediction models. With some more work involving equipment providers and the mango industry, a robust and reliable classification system that can enhance quality decisions around IDs in the mango supply chain is anticipated.

6.5 Conclusions

The demand for high-quality ‘B74’ mango fruit in export supply chains and without IDs has increased. Consequently, the need for non-destructive prediction and detection methods for IDs. Among now conventional techniques, NIR is widely used for sorting for quality and disorders in fruits. However, most NIR studies have been conducted under static laboratory conditions. This study evaluated the efficacy of in-line NIR for predicting IDs in mango fruit under near commercial conditions. While accuracy of predicting IDs based on severity rating was low, compelling accuracy (72.5%) was achieved with binary prediction. IDs were associated with fruit maturity, with less mature fruit being more prone to FCWP and more mature fruit prone to FB. In-line NIR demonstrated reasonably accurate predictions of DMC (r^2 = 0.8). Therefore, sorting mango fruit using in-line NIR

for maturity and binary classification for IDs has demonstrated potential to channel only relatively tolerant fruit into export supply chains wherein in phytosanitary treatment is requisite. Further research should improve and validate the model.

6.6 Limitation of study

The experimental investigation detailed in this chapter was carried out in collaboration with Tomra, a commercial industry based in New Zealand. In adherence to confidentiality agreements, the company opted not to disclose the entirety of the data acquired through near-infrared (NIR) scanning, sharing only the principal findings of the experiment. Additionally, a setback occurred in the second year as the NIR scanning data was inadvertently lost, rendering it impossible to incorporate second-year data into this study. Consequently, the employment of detailed chemometrics in this experiment was precluded.

6.7 References

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Chapter 7 General discussion and conclusions

7.1 Research background

'B74'(Calypso™) is the second most commercially important mango cultivar in Australia (Hort Innovation, 2020). Consumer acceptability and demand for this cultivar is high both in domestic and overseas markets (Hofman *et al.,* 2010b). However, internal disorders (IDs) expressed postharvest can result in market rejections, with adverse economic consequences (Holmes, 2019a, 2019b). The IDs include jelly seed, stem end cavity (SEC), soft nose, flesh cavity, and internal browning. More generally, IDs are an obstacle to realising value, competitiveness, and consumer confidence in Australian fresh mangoes (Hofman, 1995; Ledger, 1996).

Causal factors leading to 'B74' fruit expressing IDs have not been determined. In this context, research-based recommendations are needed to inform production of more resilient fruit and to detect potentially susceptible fruit during postharvest handling, such that they might be selectively marketed. This study aimed to identifying factors predisposing fruit to IDs and strategies to reduce IDs in 'B74' mango export supply chain.

7.2 Research findings and recommendations

Figure 7.1 summarise the outcomes of the thesis chapters and recommendations towards minimising IDs in existing commercial supply chains.

7.2.1 Internal disorders

In the wake of industry survey, monitoring trials over three seasons (Chapter 3) and controlled experiments (Chapters 4 and 6) were conducted. Unexpectedly, IDs reported previously for 'B74' mango fruit were not observed. Rather, the predominant disorders were flesh cavity with white patches (FCWP) and flesh browning (FB) (Chapter 3, Section 3.3.3.3). These IDs were defined for 'B74' mango supply chains, including to obviate potential confusion with other IDs and generic terms like 'internal breakdown', 'internal disorders', or 'flesh disorders'.

This discrepancy in the name of the IDs may be due to the different names being used to describe the same disorders and / or same name for different disorders. For example, Raymond et al. (1998a) referred to a combination of various IDs, including jelly seed, soft nose, and stem end cavity, as internal breakdown. Soft nose has be considered identical with tip pulp, yeasty or insidious fruit rot (Young 1957; Lim & Khoo 1985). Similarly, Sauco et al. (1985) described soft nose as a combination of jelly seed and stem end cavity. The symptoms of flesh breakdown described by Malo

and Campbell (1978) was considered similar to stem end cavity symptoms described by Mead and Winston (1991). Moreover, Krishnamurthy (1981) and Subramanyam et al. (1971) used the term internal breakdown to describe the spongy tissue or soft centre in 'Alphonso' mango fruit.

7.2.1.1 Flesh cavity with white patches

7.2.1.1.1 Vapour heat treatment and flesh cavity with white patches

FCWP was defined as observed in VHT fruit (Chapter 3, Section 3.3.3.3.1; Appendix 21; Chapter 4, Tables 4.4 and 4.5). Other studies have reported disinfestation heat treatment, (viz., hot water treatment (HWT) (Sharp *et al.,* 1989; Jacobi and Wong, 1992; Jacobi *et al.,* 1995a; Jacobi *et al.,* 2000; Jacobi *et al.,* 2001a; Jacobi *et al.,* 2001b; Jacobi *et al.,* 2002) and VHT (Esguerra *et al.,* 1990; Esguerrra and Lizada, 1990; Jacobi and Wong, 1992; Mitcham and McDonald, 1993) induced IDs characterised by cavities and starchy layers in the fruit mesocarp (Chapter 1, Figure 1.5 B).

7.2.1.1.2 Maturity and FCWP

FCWP in 'B74' mango fruit was negatively correlated with fruit maturity (Chapter 3, Section 3.3.3.5). Fruit having <15% dry matter content (represented as a percentage of dry weight to fresh weight; DMC) or early harvested were relatively more sensitive to VHT-induced FCWP and relative tolerance to VHT increased as fruit maturity progressed (Chapter 4, Tables 4.5 and 4.5; Chapter 6, Section 6.3.3). Several researchers also reported that less mature or early harvested mango fruit were more sensitive to VHT / HWT than more mature fruit (Quimio and Quimio, 1974; Esguerra *et al.,* 1990; Jacobi *et al.,* 2001a; Jacobi *et al.,* 2001c; Brecht *et al.,* 2020).

In certain supply chains, and as per Chapter 6, incidence of FCWP was relatively higher even in more mature fruit (>17% DMC) similar to that for less mature fruit (Chapter 6, Section 6.3.3). These findings indicate that fruit sensitivity to VHT-induced FCWP is influenced by factors beyond just maturity. Jacobi *et al.* (1995b) reported no injury in 'Kensington Pride' mango fruit with different maturities after VHT.

Further study with control of different factors, such as maturity stage and postharvest transit time and temperature conditions, may be required to prove roles of various different factors in fruit sensitivity or tolerance to disorders. Thus, fruit maturity is intimately involved in disorder severity but is not only the factor.

7.2.1.1.3 Temperature conditioning and flesh cavity with white patches

The present study provides evidence that temperature conditioning prior to VHT reduced VHT-induced FCWP in 'B74' mango fruit (Chapter 4, Table 4.5). Various researchers have identified that prior conditioning increase fruit tolerance to disinfestation heat treatment and reduces physiological disorders (Smith and Chin, 1989; Klein and Lurie, 1992; Joyce *et al.,* 1993; Joyce and Shorter, 1994; Jacobi *et al.,* 1995a; Jacobi *et al.,* 2001c; Brecht *et al.,* 2020). Conditioning was found to be more efficacious in less mature fruit (Chapter 4, Section 4.3.2.2). Prior conditioning can contribute to the improved physiological stage of the fruit. The physiological stage of fruit may be enhanced through prior conditioning. Hence, it may play a crucial role in increasing fruit tolerance to disinfestation heat treatment. Other studies also suggest that physiological stage of the fruit impacted the physiological disorders (Boag *et al.,* 1990; Mohammed and Brecht, 2002; Marques *et al.,* 2012; Marques *et al.,* 2014; Sultana *et al.,* 2021).

However, there is concern that improving the thermotolerance of fruit by prior conditioning may also enhance the thermotolerance of the target insects (Beckett and Evans, 1997). Accordingly, confirmation that adopting conditioning treatments still satisfies quarantine restrictions before such treatment can be established commercially.

7.2.1.2 Flesh browning

FB is a relatively less studied disorder in mango fruit. However, in temperate fruit like apples and pears, FB has been extensively studied (Lau, 1998; Elgar *et al.,* 1999; Ferguson *et al.,* 1999; Franck *et al.,* 2007). It has been reported that FB is linked with fruit maturity (viz., higher in more mature fruit) and storage conditions (i.e., longer storage and low O_2 level) (Elgar *et al.,* 1999; Lammertyn *et al.,* 2000; Franck *et al.,* 2007).

7.2.1.2.1 Maturity and flesh browning

In this study FB incidence and severity were positively correlated with fruit maturity at harvest (Chapter 3, Sections 3.3.3.5 and 3.3.3.6). Less mature fruit showed relatively higher tolerance to FB, tolerance decreased as maturity increased (Chapter 6, Section 6.3.3). Maturation and ripening process affect cell integrity and reduces mesocarp porosity, hindering respiratory exchange (Lammertyn *et al.,* 2003a; Lammertyn *et al.,* 2003b). These changes were relatively more in the inner mesocarp of mature fruit, as indicated by higher T2 and PD values in MR images (Chapter 5, Section 5.3.1.1 and 5.3.1.2), during the early stages of ripening. This could explain why more mature fruit are more prone to FB.

In an experiment (Chapter 4, Sections 4.3.1.1 and 4.3.2.2), with more mature fruit (>17% DMC), there was no FB. This suggests that FB in 'B74' mango is not solely dependent on fruit maturity and that variables other than maturity also impact on fruit susceptibility to FB.

7.2.1.2.2 Postharvest time and temperature, and flesh browning

Postharvest handling, particularly postharvest time and temperature may play a role in FB development (Chapter 3, Section 3.3.3.6, Table 3.5). Several studies suggested FB in mango fruit is a function of these postharvest conditions (Hofman *et al.,* 2010a; Brecht *et al.,* 2012; Miguel *et al.,* 2013; Brecht *et al.,* 2019; Brecht *et al.,* 2020; Costa *et al.,* 2021). Chapter 4 utilised fruit from the one orchard block for three experiments with similar procedures except for treatment allocation. In experiment 3, fruit underwent a 21-day sea freight simulation, while in the other experiments fruit were stored at room conditions after VHT. However, FB occurrence was limited to experiment 3 (Chapter 4, Section 4.3.3.2). A processor survey also revealed similar disparity in that a sea freighted consignment had FB, while air freighted consignments had no FB (Chapter 3, Section 3.3.2.1).

Furthermore, monitoring trials revealed that fruit from the same orchard block, but with subsequently different supply chains, exhibited varying degrees of FB incidence (Chapter 3, Table 3.2, and Figure 3.3). The differences noted were postharvest handling time and temperature. Similarly, FB was observed in domestic supply chains handled by one ripening centre, while fruit handled by other ripening centres were of good quality (Chapter 3, Section 3.3.2.1). These results provide compelling evidence that postharvest handling operations can render fruit susceptible to FB. This represents opportunity for further investigation the association of different factors beyond maturity.

7.2.1.2.3 Vapour heat treatment and flesh browning

It was hypothesised that FB in mango fruit is promoted by disinfestation heat (Esguerra *et al.,* 1990; Esguerrra and Lizada, 1990; Mitcham and McDonald, 1993). The hypothesis that fruit turns brown after heat disinfestation (VHT) could not be definitively established from this study because FB was observed in fruit irrespective of VHT (Appendices 23, 24 and 25).

7.2.1.3 Fruit nutrient content and internal disorders

It was also hypothesised that the high-level of N and insufficient Ca application by growers could contribute to IDs in mango (Young and Miner, 1961; Raymond *et al.,* 1998b; Whangchai *et al.,* 2001; Torres *et al.,* 2004; Bally, 2006; Ma *et al.,* 2018). However, grower surveys revealed that balanced nutrient with adequate Ca levels were being applied (Appendix 11). Notwithstanding, there

was a lot of variation in incidence and severity between two growers with similar nutrient management (Chapter 3, Tables 3.1 and 3.2). Furthermore, wide variation in incidence and severity of disorders in different supply chains from the same orchard block and assumed to have similar nutrient content harvested at different times (Chapter 3, Figure 3.3 and 3.4, and Appendices 15 and 18) tends not support an association between nutrient imbalances and IDs. Hence, while balanced nutrients may make the fruit more robust and tolerant to different stresses and disorders, high N and low Ca may not be the primary causes of the IDs in question.

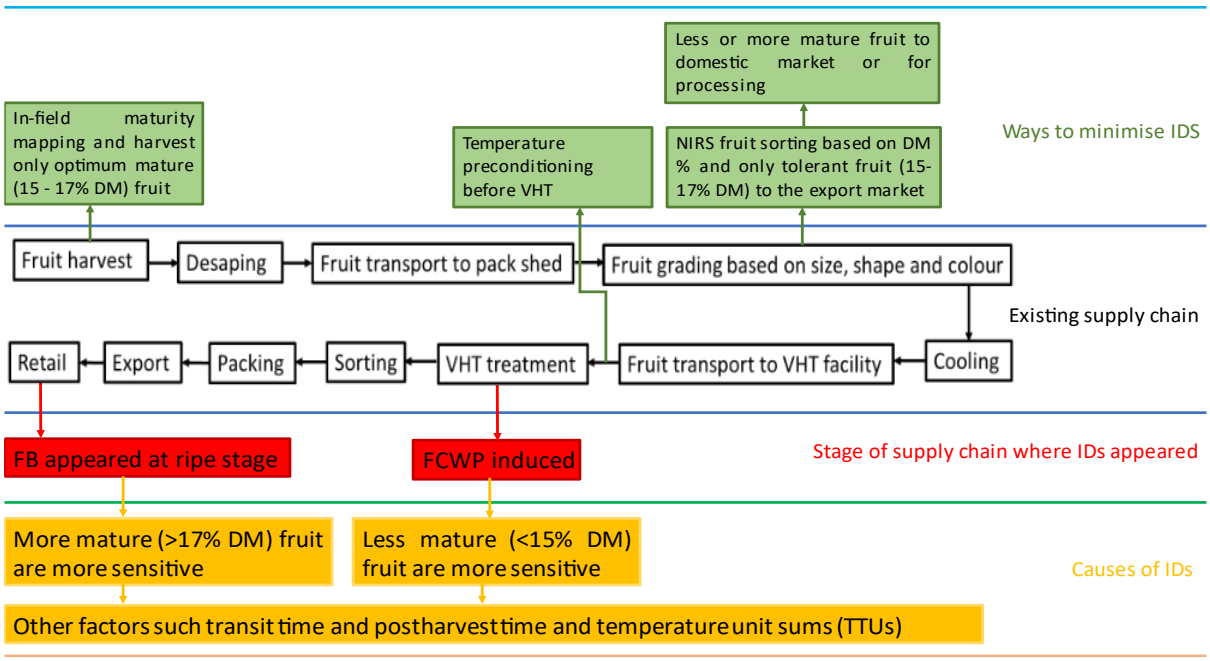


Figure 7.1: Diagrammatic model linking the outcomes of the thesis chapters in the existing supply chain. This model visually depicts the specific stages within the supply chain where internal disorders (IDs) under investigation are observed. Furthermore, the model elucidates the primary causes of these IDs and proposes strategies to mitigate and minimise such disorders within the existing supply chain.

7.3 Practical implication of the research outcomes

Presence of a range in maturity levels in a single harvest (Chapter 3, Section 3.3.3.2, and Figure 3.4) is a common problematic phenomenon (Ainsworth, 2016). Figure 7.2 illustrates the strategy to address the DMC variation within a single harvest. Maturity mapping of the orchard and harvesting fruit based on their maturity zones could lead to a more homogenous and desirable maturity range in a single harvest (Hofman and Whiley, 2010). Felix Instruments, USA in association with Central Queensland University, Australia developed an online dashboard (https://fruitmaps.com/) to map fruit maturity across the orchard. Such information allows

identification of different rates of fruit maturation and provides guidance for scheduling the order of harvests across an orchard (Subedi *et al.*, 2013).

Even after implementing in-field maturity mapping, there will be a heterogeneous mixture of fruit maturities within a harvest. In this context, non-destructive fruit sorting using in-line NIR can effectively predict DMC (r^2 = 0.8, SEP = 0.7) and FCWP with 72.5% accuracy (Chapter 6, Section 6.3.4). This practice should help for selectively marketing relatively tolerant fruit to minimise IDs in the market.

In order to implement a long-term strategy (Figure 7.3), occurrence of IDs at the farm or block level can be determined by regular monitoring of IDs within the supply chains. Identification and mapping of IDs back to farm and block levels allows for thorough assessment of their spatial and temporal patterns. This enables identification of best and worst-performing blocks under various conditions. By analysing factors contributing to low-quality fruit in the supply chains, best performing blocks can be maintained for high-quality fruit, while agronomic practices can be managed to improve performance of worst-performing blocks.

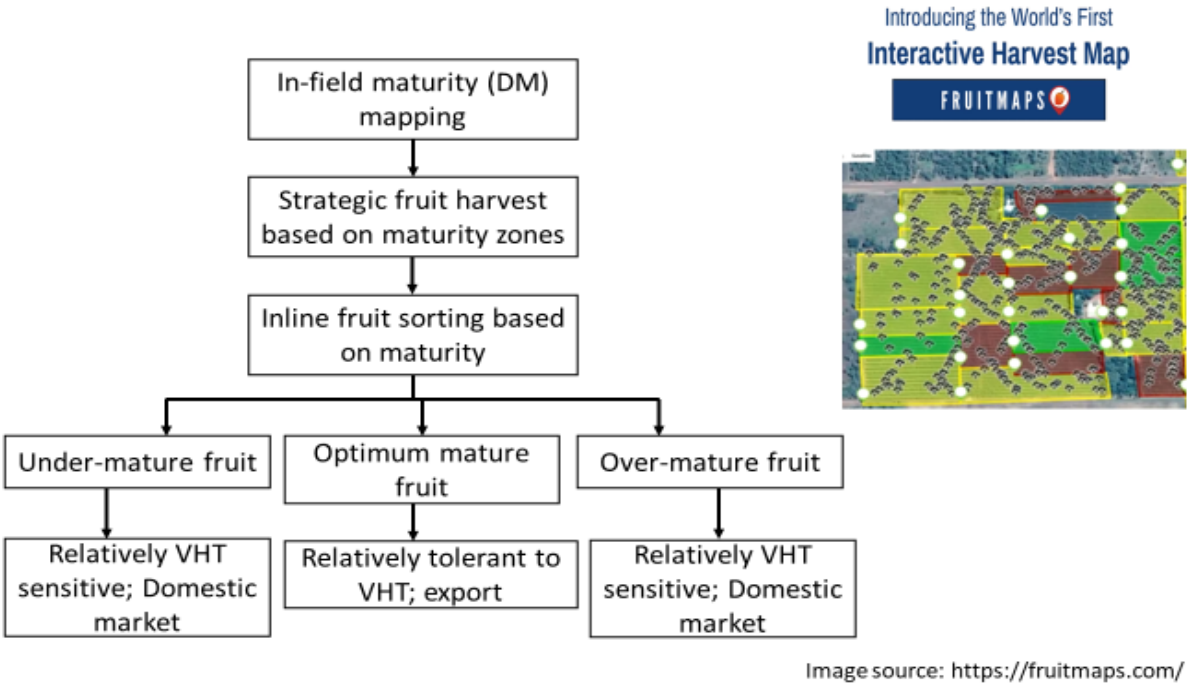


Image source: https://fruitmaps.com/

Figure 7.2: Strategy to reduce internal disorders (IDs) of 'B74' mango fruit in export supply chain.

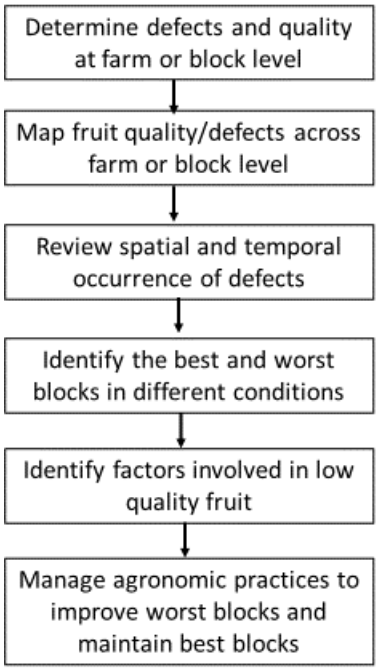


Figure 7.3: Long-term strategy to reduce internal disorders (IDs) of 'B74' mango fruit.

7.4 Limitations and challenges

Throughout the course of the research, an inherent challenge emerged in obtaining comprehensive insight into growers' management practices. The intention was to conduct surveys with growers and then fruit handlers in order to inform controlled experimentation. However, the onset of the COVID-19 pandemic and producer reticence hindered data collection efforts, impeding movement, and limiting experiential insight.

The studied disorders result from multiple factors rather than a single cause. Understanding this requires comprehensive documentation of crop pre- and post-harvest conditions. However, growers constrained by time and inadequate record-keeping of management practices, encountered challenges in achieving comprehensive documentation.

Monitoring studies conducted in Chapter 3 presented several noteworthy challenges. 'B74' mango fruit which were exported originated from growing regions in the Northern Territory and North Queensland, situated at considerable distances of *ca.* 3424 and *ca.* 1720 km, respectively, from the VHT facility. An objective during the monitoring phase was to monitor and estimate postharvest time-temperature units (TTUs) to arrival at the VHT facility. Unfortunately, growers were unable to comply. Hence, potentially valuable information, including temperature at harvest, time taken from

harvest to the packing shed, packing shed temperature, and time spent in the packing shed, were unavailable. General issues were data loggers were inadvertently omitted from trucks or failed to accurately log the data. Such unforeseen circumstances impacted the reliability and comprehensiveness of the monitoring process.

Lastly, seasonal variations on IDs incidence (Chapter 4) somewhat limited thorough investigation of maturity linkage to IDs.

7.5 Direction for future research

- The present study surveyed only the two ‘B74’ growers who sent fruit for export. Likewise, it used fruit only from these two growers for monitoring trials. It is recommended to expand the scope of study to more ‘B74’ growers. Additionally, repeat monitoring of supply chains (Figure 7.3) should be conducted, encompassing fruit from multiple sources towards better understanding causes and controls of IDs.

- In a fully integrated context, further studies based on monitoring and modelling from preharvest through postharvest should empower relatively precise management and minimisation of IDs in ‘B74’ supply chains. This is because expression of physiological disorder incidence and / or severity can vary with environmental and / or management practices, including temperatures during fruit maturation, harvest maturity, and plant nutrition, irrigation, and pruning, for example. Overall, developing understanding of the genotype × environment × management interaction (G × E × M) for both pre- and postharvest paddock to plate phases is important.

- The present study identified the pivotal relationship between fruit maturity and IDs. However, and while other factors such as transit time, postharvest TTUs were identified, their influences were not tested in this relatively limited study. Further investigation in controlled experiments is warranted to qualify and quantify these other determining factors with fruit at different maturities.

- A comprehensive investigation into the physiological and biochemical aspects of IDs should enhance understanding underlying mechanisms and may identify additional factors that influence fruit susceptibility to IDs. Comparing physiological and biochemical characteristics of ‘B74’ mangoes with those of tolerant varieties could provide deeper insights into mechanisms responsible for variations in tolerance across cultivars.

7.6 Research contribution

The present study has provided compelling evidence that fruit maturity at harvest for ‘B74’ mango is a crucial determinant of the IDs. Other probable deterministic factors evidently include transit time prior to VHT and postharvest TTUs. In-field maturity mapping and scheduled harvesting based on maturity zones to obtain optimally mature fruit (15 - 17% DMC). Additionally, an in-line NIR sorter can be utilised for maturity, enabling selective marketing of IDs tolerant fruit. Thereafter, optimisation of postharvest storage conditions. Growing the knowledge base will ultimately realise optimum quality of harvested fruit and attendant post-harvest practices to cement consumer trust and ensure repeat purchases. Such measures should also increase grower profits and support sustainability of the ‘B74’ mango industry.

7.7 References

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Appendices

Appendix 1: NIRS used in quality detection of different fruit and vegetables.

Fruit/vegetable	Wavelength band (nm)	Quality attribute	Spectral acquisition mode	Regression model	R^2 of regression model	Reference
Mango	684 - 990	DM		PLSR	0.88	(Anderson *et al.,* 2020)
				ANN	0.89	
	400 - 700	TSS		PLSR	0.83-0.87	(Cortés *et al.,* 2016)
	600 - 1100	Firmness			0.87-0.89	
	900 - 1750	Flesh colour			0.81-0.83	
	306 - 1140	SSC	Interactance	PLSR	0.87	(dos Santos Neto *et al.,*
		DM			0.84	2017)
	300 - 1100	Internal browning	Reflectance	ANN		(Gabriëls *et al.,* 2020)
				PLSR		
	400 - 1100	Jelly seed	Interactance	Logistic	0.71	(Mogollón *et al.,* 2020)
		Black flesh		LDA	0.76	
	400 - 700	TSS	Reflectance	MLR	0.90	(Jha *et al.,* 2005)
	400 - 700	Firmness	Reflectance	MLR	0.87	(Jha *et al.,* 2006)
				PCR	0.87	
		Flesh colour		MLR	0.95	
				PCR	0.95	
	1200 - 2200	TSS	Diffuse reflectance	MLR	0.76	(Jha *et al.,* 2012)
		pH		PLSR	0.70	

Sweet cherry	729 - 975	SSC	Interactance	PLSR	0.92-0.94	(Escribano *et al.*, 2017)
					0.91-0.93	
		DM				
Pomegranate	800 - 2500	TSS			0.87	(Arendse *et al.*, 2017)
		pH			0.85	
		TA			0.85	
		Vit C			0.84	
		Firmness			0.68	
Passion fruit	800 - 2700	SSC	Interactance	PLSR	0.63	(de Oliveira *et al.*, 2014)
		TA			0.49	
Tomato	800 - 2700	SSC	Interactance	PLSR	0.52	(de Oliveira *et al.*, 2014)
		TA			0.51	
Apricot	800 - 2700	SSC	Interactance	PLSR	0.93	(de Oliveira *et al.*, 2014)
		TA			0.95	
Apple	700 - 900	Brownheart	Transmission	MLR	0.69	(Clark *et al.*, 2003)
				PLSR	0.91	
	325 - 1075	Variety	Reflectance	PCA	0. 98	(He *et al.*, 2007)
		classification		ANN	1	
	935 - 2500	Bitter pit	Reflectance	PLSR	1	(Jarolmasjed *et al.*, 2017)
	350 - 2500	Bitter pit	Reflectance	SMLR	0.78-0.87	(Kafle *et al.*, 2016)
		Internal flesh	Interactance	PLSR	0.83	(Khatiwada *et al.*, 2016)
		browning	Partial transmission			

			Full transmission			
	100 - 1100	Internal browning				(Mogollon *et al.,* 2020)
	650 - 950	Internal browning				(McGlone *et al.,* 2005)
	550 - 1650	Internal defect				(Huang *et al.,* 2020)
Pear	400 - 1028	Brown heart	Transmission		0.91	(Fu *et al.,* 2007)
	670 - 1110 and 800 - 2630	Brown heart	Reflectance		0.80 0.75	(Fu *et al.,* 2007)
	651 - 1228	Brown core	Transmittance		0.95	(Han *et al.,* 2006)
Rockmelon	695 - 1045	TSS	Partial transmittance	MPLSR	0.56-0.91	(Guthrie *et al.,* 2006)
Melon	700 - 1100	TSS	Reflectance		0.82	(Guthrie *et al.,* 1998)
Pineapple	700 - 1100	TSS	Reflectance		0.65	(Guthrie *et al.,* 1998)
Mandarin	306 - 1130	TSS	Interactance		>0.75	(Guthrie *et al.,* 2005)
		DM			>0.90	
Kiwifruit		TSS	Reflectance			(Schaare and Fraser, 2000)
		Density	Interactance			
		Pulp colour	Transmittance			

PLSR: Partial least square regression, ANN: Artificial neural network, MLR: Multiple linear regression, PCR: Principal component regression, SMLR: Stepwise multilinear regression, PCA: Principal component analysis, R^2: Coefficient of determination

Appendix 2: Hyper and multispectral imaging used in quality detection in different fruit and vegetables.

Fruit/vegetable	Wavelength band (nm)	Quality attribute	R^2	Reference
Citrus	320 - 1100	Green mould affected	0.91	(Gómez-Sanchis *et al.*, 2008)
	730 - 830	Canker	0.85	(Qin *et al.*, 2008)
Apple	550 - 1650	Internal defect	>0.89	(Huang *et al.*, 2020)
Banana	400 - 1000	SSC	0.85	(Rajkumar *et al.*, 2012)
		Moisture content	0.87	
		Firmness	0.91	
Nanfeng Orange	700 - 1100	SSC	0.99	(Liu *et al.*, 2007)
Navel Orange			0.96	
Orange	400 - 1000	Bruising	0.93	(Li *et al.*, 2011)
Mango	380 - 1000	SSC	0.79	(Makino *et al.*, 2012)
Pomegranate	700 - 1000	TSS	0.94	(Khodabakhshian *et al.*, 2017)
		pH	0.88	
		TA	0.93	
Water melon	400 - 1100	SSC	0.89	(Sugiyama and Tsuta, 2010)

Appendix 3: X-ray CT used in quality detection in different fruit and vegetables.

Fruit/vegetable	Quality attribute	Reference
Pomegranate	Volume estimation of internal fractions	(Arendse *et al.*, 2016b)
	Density, False codling moth infestation, and Blackheart	(Arendse *et al.*, 2016a)
	Internal structure	(Magwaza and Opara, 2014)
Mango	Microstructure of mango	(Cantre *et al.*, 2014)

	Internal and external morphological characteristics	(Jha *et al.*, 2010)
Apples	Water core	(Herremans *et al.*, 2014b)
	Browning	(Herremans *et al.*, 2013)
	Pore space in apples	(Mendoza *et al.*, 2007)
	Bruising	(Shahin *et al.*, 1999; Shahin *et al.*, 2002)
Pear	Core breakdown	(Lammertyn *et al.*, 2003a; Lammertyn *et al.*, 2003b)

Appendix 4: MRI technology used in quality detection of different fruit and vegetables.

Fruit/vegetable	Quality attribute	Reference
Apple	Mealiness	(Barreiro *et al.*, 1999; Barreiro *et al.*, 2000; Marigheto *et al.*, 2008)
	Internal browning	(Jung *et al.*, 1998; Clark and Burmeister, 1999; Gonzalez *et al.*, 2001; Chayaprasert and Stroshine, 2005; Cho *et al.*, 2008)
	Water core	(Wang *et al.*, 1988; Cho *et al.*, 2008; Melado-Herreros *et al.*, 2013)
	Water core	(Herremans *et al.*, 2014b)
	Tissue characterisation	(Defraeye *et al.*, 2013)
	Infestation of peach fruit moth	(Haishi *et al.*, 2011)
	SSC	(Keener *et al.*, 1999)
	Bruising	(McCarthy *et al.*, 1995)
Peach	Mealiness	(Barreiro *et al.*, 2000)
Avocado	Maturity	(Chen *et al.*, 1996)
Persimmon	Chilling injury	(Clark and Forbes, 1994)
Citrus	Ripening and decay	(Galed *et al.*, 2004)
Pear	Internal browning	(Hernández-Sánchez *et al.*, 2007)

	Water profile	(Nguyen *et al.*, 2006)
	Water content and Tissue damage	(Suchanek *et al.*, 2017)
	Core breakdown	(Wang and Wang, 1989; Lammertyn *et al.*, 2003a; Lammertyn *et al.*, 2003b)
Mango	Water activity	(Joyce *et al.*, 2002)
	Heat treatment injury	(Joyce *et al.*, 1993)
Kiwifruit	Structural changes	(Taglienti *et al.*, 2009)
Strawberry	Damage due to pressure	(Otero and Préstamo, 2009)
Tomato	Pericarp damage	(Milczarek *et al.*, 2009; Musse *et al.*, 2009)
	Ripening	(Musse *et al.*, 2009)
Cucumber	Freezing damage	(Kotwaliwale *et al.*, 2012)
Potato	Internal bruise	(Thybo *et al.*, 2004)
	Sparing disease	
Melon	Tissue breakdown	(Zion, 1994)
Olive	Bitter pit	(Zion *et al.*, 1997)

Appendix 5: Acoustic technology in quality detection of different fruit and vegetables.

Fruit/vegetable	Quality attribute	R^2	Reference
Mango	Firmness	> 0.96	(Al-Haq and Sugiyama, 2004)
Apple	Firmness	< 0.27	(Armstrong *et al.*, 1990)
	Mealiness		(Andani *et al.*, 1999)

Watermelon	Hollow heart		(Stone *et al.*, 1996)
	Mesocarp colour		
	Sugar content		
	Firmness		
Melon	SSC	0.96	(Sakurai *et al.*, 2014)
Tomato	Chilling injury		(Verlinden *et al.*, 2004)
Peach	Split pit	> 0.75	(Kawai *et al.*, 2018; Nakano *et al.*, 2018)
Kiwifruit	Firmness	0.90	(Sakurai *et al.*, 2014)
Pear	Core rot	0.96	(Kadowaki *et al.*, 2012)
	Core rot	0.96	(Sakurai *et al.*, 2014)
	Firmness	0.80	(Oveisi *et al.*, 2014)
Swede	Brown heart	0.67	(Mohd, 2016)

Appendix 6: Survey form for Calypso mango growers

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

TO ASSESS THE CAUSES OF THE INTERNAL DISORDERS OF 'CALYPSO' MANGO

The purpose of the survey is to identify the causes of the internal disorders of Calypso™ mango. The response will be used by the UQ Ph.D. students involved in the CRC NA project with Perfection Fresh. The research is aimed to reduce the commercial losses due to flesh disorders. The responses collected will be confidential and not shared.

Contact: Dr. Daryl Joyce (Project Leader); M: 0428867804; Email: d.joyce@uq.edu.au

Date:

Region: Name of the farm:

Objective:

- To assess the causes of the internal disorder

1. Area of farm:
2. How old is plantation:
3. Planting density:
4. Rootstock:
5. Site location:
6. Environment: (data from the weather station from each block)

Temperature	Rainfall	Humidity	VPD

7. Soil characteristics of the farm (soil test report of the season)

Block	Soil type	pH	Electrical conductivity (EC)	Cation exchange capacity (CEC)

8. Irrigation (If differs according to block please mention it)

Block	Amount	Frequency	Schedule on what basis	Irrigation system	Regulation of irrigation before harvest/any stages

9. Weed control (If differs according to block please mention it)

Method (s)	Frequency	Concentration (if chemicals)

10. Nutrient management

Nutrients	Source of nutrient	Time of application (mention if splitting is done)	Amount/tree	Amount/Acre

11. Disease control (If differs according to block please mention it)

Disease(s)	Control means	Frequency of treatment application	Concentration of chemical (s)

12. Pest control (If differs according to block please mention it)

Pest(s)	Control means	Frequency of treatment application	Concentration of chemical (s)

13. Pruning (If differs according to block please mention it)

When (months)	Level of pruning (heavy, mild, low)	What type of branches	Frequency per year

14. Time of flowering

Blocks	Months

15. Estimated date of harvest

Blocks	Estimated month of harvest	Way of determining when to harvest (e.g. % DM, flesh colour, heat sums etc.)	Harvest on which stage? e.g. at what dry matter level/what flesh colour/what heat sums?	What aids used for harvesting?

16. Postharvest treatment (e.g. desapping, fungicide treatment etc)

Chemical(s)/PGR (s)/ physical	Concentration/Temperature	When	Duration

17. Typical time from harvest to pack shed……..?

18. Sorting and Grading

Basis of sorting/grading	How many grades	Technology used for sorting/grading

19. Cooling

Cooling method (s)	Target temperature	Typical time to reach the required temperature

20. Holding conditions before dispatch

Types of storage e.g. room storage, temperature controlled storage etc.)	Target temperature	RH	Typical duration of holding

21. Transport

Means of transport	Target temperature for transport	Monitoring of temperature during transport?	Typical hours from farm to agent

22. Typical time from pack shed to VHT facility…….?

23. What skin disorders have you recorded during the packing and sorting process, or reject bin analysis?

What	Severity	Year/Season	Probable cause

24. What flesh disorders have you recorded during the packing and sorting process, or reject bin analysis?

What	Severity	Year/Season	Probable cause

Thank You

Appendix 7: Survey form for VHT facility

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

TO ASSESS THE CAUSES OF THE INTERNAL DISORDERS OF 'CALYPSO™' MANGO

The purpose of the survey is to identify the causes of the internal disorders of Calypso™ mango. The response will be used by the UQ Ph.D. students for the thesis. The responses collected will be confidential and anonymity of the respondent.

Contact: Dr. Daryl Joyce (Project Leader)
M: 0428867804
Email: d.joyce@uq.edu.au

Date:

Objective:

- To assess the causes of the internal disorder

Please add more space (row or column) if given space is not sufficient

1. After how many hours of harvest VHT is started?

Region	Farm	Harvest date	VHT date	Hours difference between harvest and VHT

2. Is there delays/waiting in the VHT facility after the arrival of fruits? If yes

Held at what temperature?	For how long?

3. Are there any treatments done before VHT? If yes

What treatment (s)?	Duration/Concentration	Purpose	Beneficial/harmful for internal/ external disorders?

4. Are there any treatments done after VHT? If yes

What treatment (s)?	Duration/Concentration	Purpose	Beneficial/harmful for internal/ external disorders?

5. Ripening (If different practices were followed please mention with additional rows; please include past experience if any)

How	When	Is there any difference in fruit quality with different ripening practices and time? If yes mention

6. Packaging

Types of package	Number of fruits per package

7. Holding before dispatch

Temperature of holding room	RH	Gaseous composition	Duration

8. Transport to export markets

Means of transport	Temperature in transport	Humidity in transport	Gases in transport	Transport hour from VHT to importer

9. External Disorder

Disorders	Severity	Year/season	Fruit from which farm?

10. Internal disorder

Disorders	Severity	Year/season	Fruit from which farm?

11. Please mention any related information to disorders of Calypso^{TM} mango ……..

12. Any suggestions regarding the cause and reduction of internal disorder of Calypso^{TM} mango……

Thank You

Appendix 8: Ethics approval

THE UNIVERSITY OF QUEENSLAND

Institutional Human Research Ethics Approval

Project Title:	Improving production practices and non-destructive assessment to reduce the incidence of internal disorders in 'CalypsoTM' mango fruit
Chief Investigator:	Dr Daryl Joyce
Supervisor:	Dr Daryl Joyce, Dr Peter Hofman, Dr Andrew Macnish, Mr Peter Delis
Co-Investigator(s):	Mr Muhammad Asad Ullah, Mr Amit Khanal
School(s):	School of Agriculture and Food Sciences, The University of Queensland
Approval Number:	2020001261
Granting Agency/Degree:	CRCNA and Perfection Fresh / DAF and UQ / PhD
Duration:	31 July 2023

Comments/Conditions:

- HREA Form, 23/06/2020
- Consent form, 23/06/2020
- Participant Information Sheet for Growers, 23/06/2020
- Participant Information Sheet for VHT DJ, 23/06/2020
- Project Description, 23/06/2020
- Survey for Calypso mango growers, 23/06/2020
- Survey form for VHT facility, 23/06/2020

Note: if this approval is for amendments to an already approved protocol for which a UQ Clinical Trials Protection/Insurance Form was originally submitted, then the researchers must directly notify the UQ Insurance Office of any changes to that Form and Participant Information Sheets & Consent Forms as a result of the amendments, before action.

Name of responsible Sub-Committee:
University of Queensland Science, Low & Negligible Risk Ethics Sub-Committee
This project complies with the provisions contained in the *National Statement on Ethical Conduct in Human Research* and complies with the regulations governing experimentation on humans.

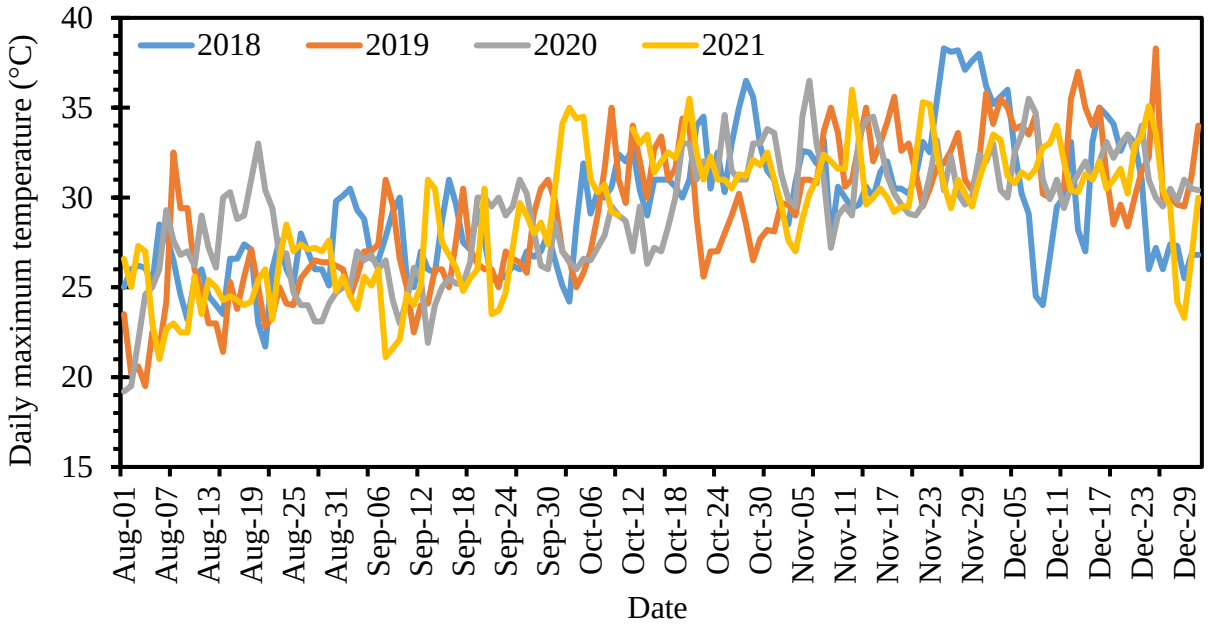
Name of Ethics Sub-Committee representative:
Associate Professor Jack Wang
Acting Chairperson
University of Queensland Science, Low & Negligible Risk Ethics Sub-Committee

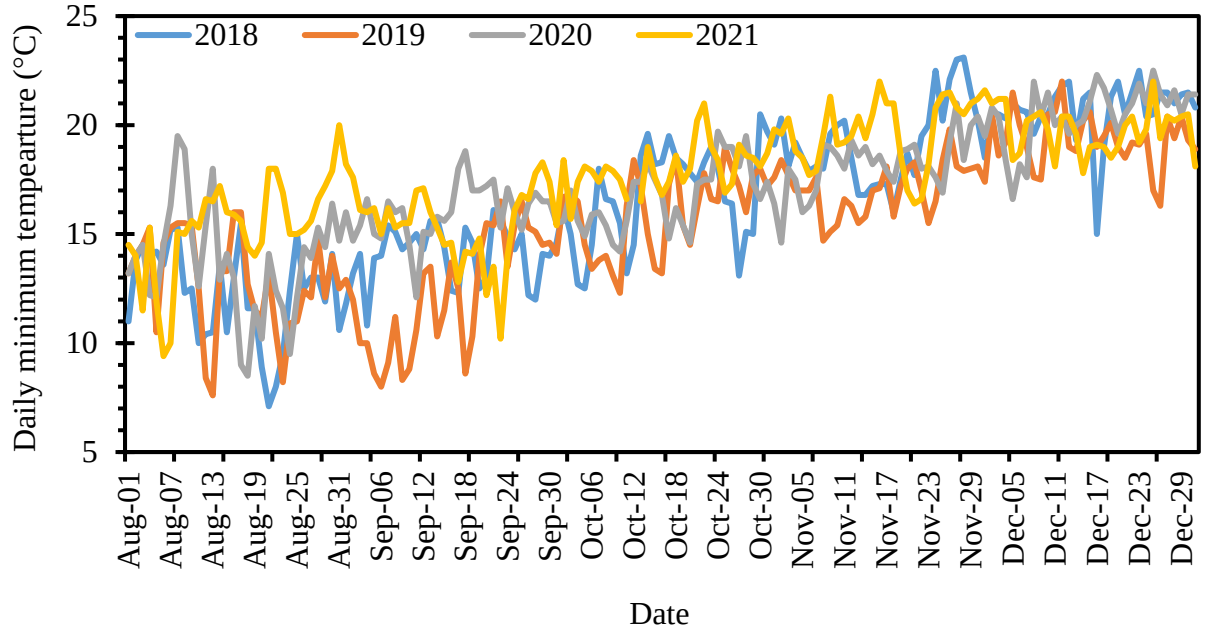
Signature _____ Date 29/06/2020

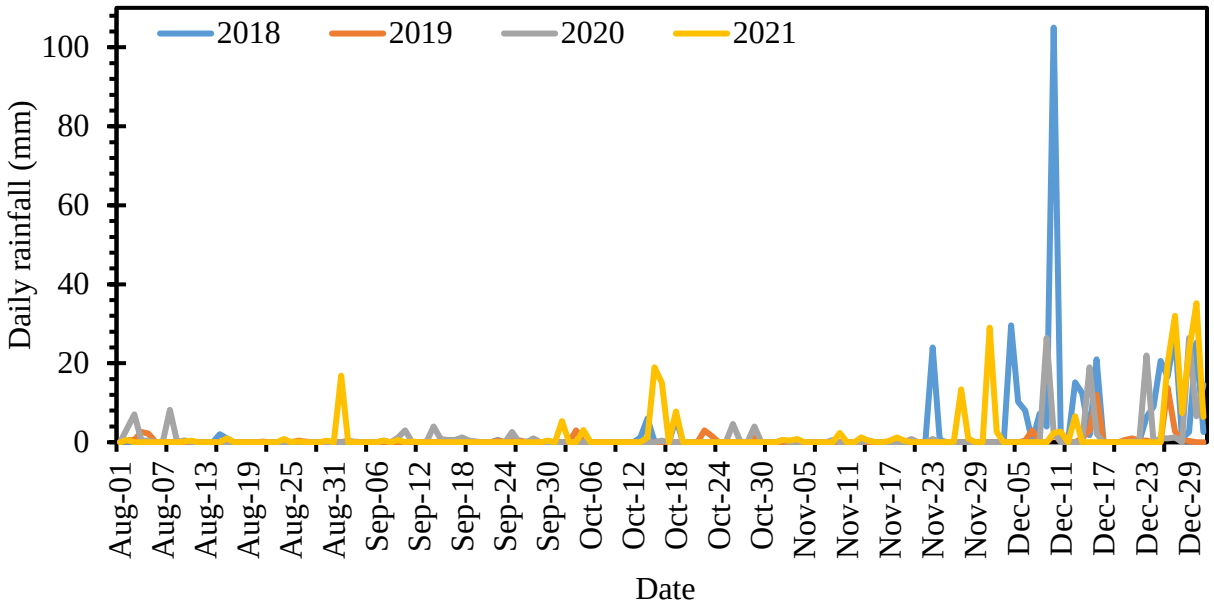
Appendix 9: Rating scale for flesh cavity with white patches (FCWP) and flesh browning (FB) based on severity. Images on left panel show the rating scale for FCWP, and the right panel shows the rating scale for FB. Both disorders were rated on a 0 - 3 scale; 0: healthy, 1: slight, 2: moderate, and 3: severe.

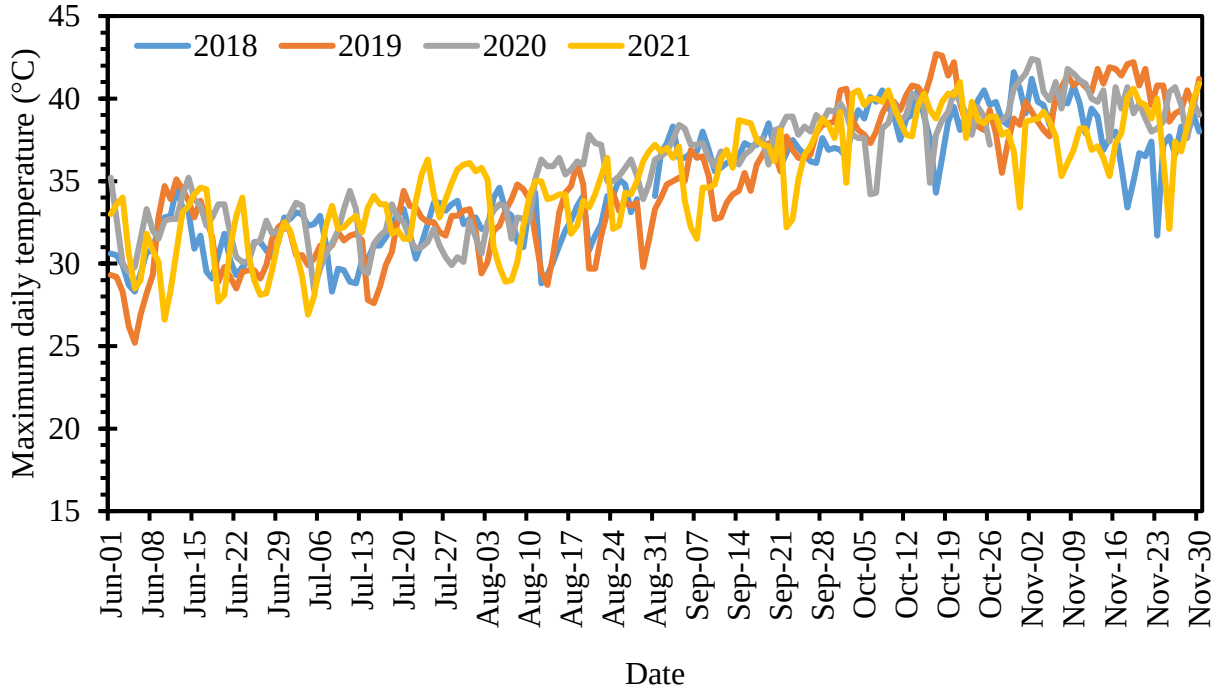
Different severity of FCWP	Rating and description	Different severity of FB	Rating and description
	0: Healthy; no signs of cavity and starchy lesions in mesocarp.		0: Healthy, no sign of brown discolouration in mesocarp.
	1: Slight; >0 -<25% area of mesocarp that cover the seed affected or any part of mesocarp.		1: Slight, >0 - <25% area of mesocarp with brown discolouration.
	2: Moderate: moderate, >25 - <50% area of mesocarp that surrounds seed affected.		2: Moderate, >25 - <50% area of mesocarp with brown discolouration.
	3: Severe, >50% area of mesocarp that surrounds seed affected		3: Severe, >50% area of mesocarp with brown discolouration

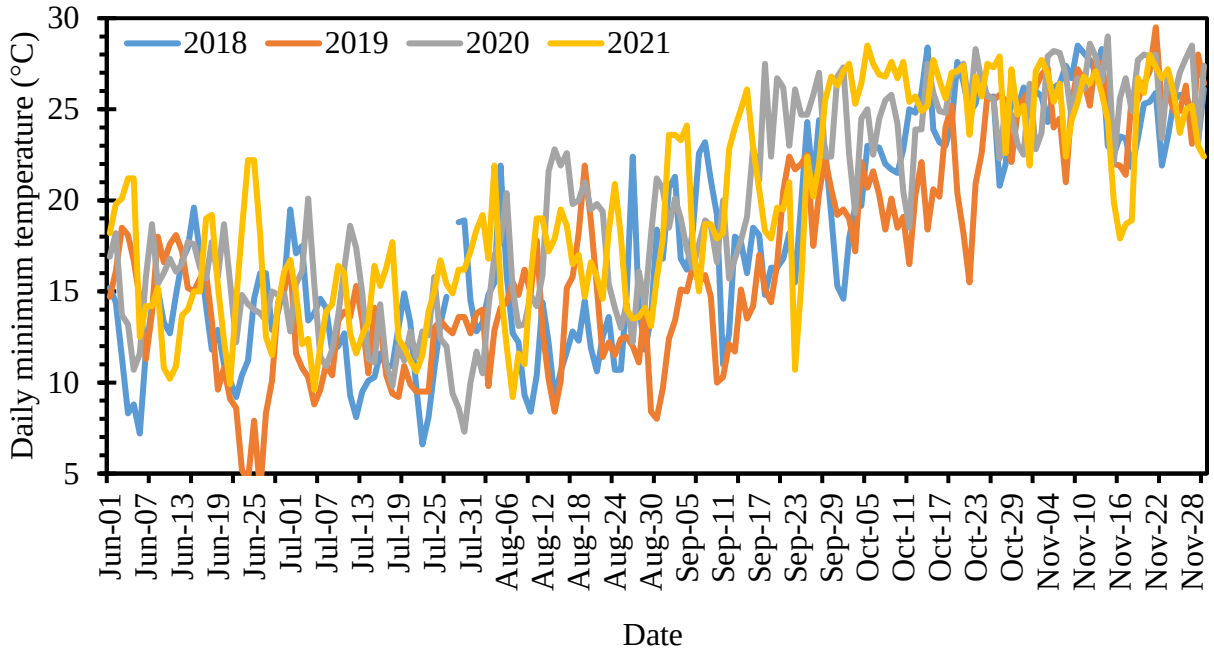
Appendix 10: Daily maximum temperature (°C), daily minimum temperature (°C) and daily rainfall (mm) of the area near 'B74' mango farm from 2018 to 2021 during flowering to fruit harvest period. Export of 'B74' mangoes involved only two growers, one located in Northern Territory (NT) and the other in North Queensland (NQ), Australia. Upper three graphs are the daily maximum and minimum temperature, and daily rainfall, respectively for the NQ farm and lower three graphs are for the NT farm. Meteorological information for each farm was collected from the nearest station of Australian Government Bureau of Meteorology (BOM; http://www.bom.gov.au/climate/averages/tables/ca_qld_names.shtml). Data from Tindal Raff (Station number: 014932) and Walkmin Research Station (Station number: 031108) were used for NT and NQ farm, respectively.

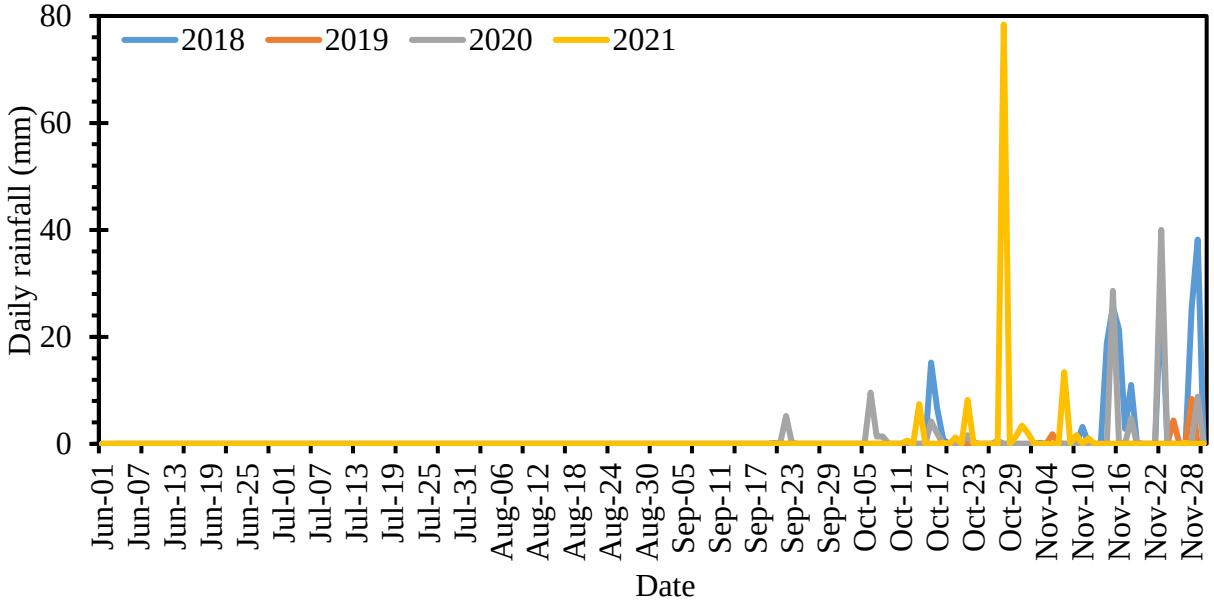





2018
2019
2020
2021
Daily rainfall (mm)
0
20
40
60
80
100
Aug-01
Aug-07
Aug-13
Aug-19
Aug-25
Aug-31
Sep-06
Sep-12
Sep-18
Sep-24
Sep-30
Oct-06
Oct-12
Oct-18
Oct-24
Oct-30
Nov-05
Nov-11
Nov-17
Nov-23
Nov-29
Dec-05
Dec-11
Dec-17
Dec-23
Dec-29
Date


2018
2019
2020
2021
Maximum daily temperature (°C)
15
20
25
30
35
40
45
Jun-01
Jun-08
Jun-15
Jun-22
Jun-29
Jul-06
Jul-13
Jul-20
Jul-27
Aug-03
Aug-10
Aug-17
Aug-24
Aug-31
Sep-07
Sep-14
Sep-21
Sep-28
Oct-05
Oct-12
Oct-19
Oct-26
Nov-02
Nov-09
Nov-16
Nov-23
Nov-30
Date


2018
2019
2020
2021
Daily minimum temperature (°C)
30
25
20
15
10
5
Jun-01
Jun-07
Jun-13
Jun-19
Jun-25
Jul-01
Jul-07
Jul-13
Jul-19
Jul-25
Jul-31
Aug-06
Aug-12
Aug-18
Aug-24
Aug-30
Sep-05
Sep-11
Sep-17
Sep-23
Sep-29
Oct-05
Oct-11
Oct-17
Oct-23
Oct-29
Nov-04
Nov-10
Nov-16
Nov-22
Nov-28
Date


2018
2019
2020
2021
Daily rainfall (mm)
80
60
40
20
0
Jun-01
Jun-07
Jun-13
Jun-19
Jun-25
Jul-01
Jul-07
Jul-13
Jul-19
Jul-25
Jul-31
Aug-06
Aug-12
Aug-18
Aug-24
Aug-30
Sep-05
Sep-11
Sep-17
Sep-23
Sep-29
Oct-05
Oct-11
Oct-17
Oct-23
Oct-29
Nov-04
Nov-10
Nov-16
Nov-22
Nov-28
Date

Appendix 11: Nutrient management plan adopted by a 'B74' mango grower who exports 'B74' mangoes. Export of 'B74' mangoes come from only two growers in Northern Territory (NT) and in North Queensland (NQ), respectively. The 'baseline' data presented were collected in 2022 and reflect nutrient management practices over three years.

Region	Nutrient source	Available nutrients	Number of applications	Application month / stage	Application rate	N and Ca applied per tree (g or ml)
NT	Horti Trace	Mg: 2%, Zn-2.5%, Mn:3%, S:6.2%, Cu: 5%, Fe: 1%, B: 0.04%, and Mo: 0.05%	1	Jul: just before flowering	5 litres / ha	-
	CK 555	N: 12.8%, P: 14.2%, K: 11.9%, and S:6.4%	1	Dec: after harvest	1.3 kg / tree	N: 166 g
	Encal	N: 13%, Ca: 16%, Mg: 1.7%, Fe: 0.12%, B: 0.8%, Zn: 0.06%, Cu: 0.06% and Mn: 0.06%	5 (foliar)	Jul-Nov (1 application in each month)	2.4 litres / tree	N: 312 ml and Ca: 384 ml
	BioGyp	Ca: 20% and S: 15.31%	1	Jul: just before flowering	300 ml / tree	Ca: 60 ml
	Thio K	K: 30% and S: 25%	2 (fertigation)	Jul and Sep	186 ml / tree	-
		Total N and Ca applied/tree				N: 478 and Ca: 444
NQ	Nitrophoska	N: 12%, P: 5.2%, K: 14.1%, S: 8%, Ca: 3.2%, Mg: 1.2%, B: 0.02%, and Zn: 0.01%	1	Apr	3.2 kg / tree	N: 384 g

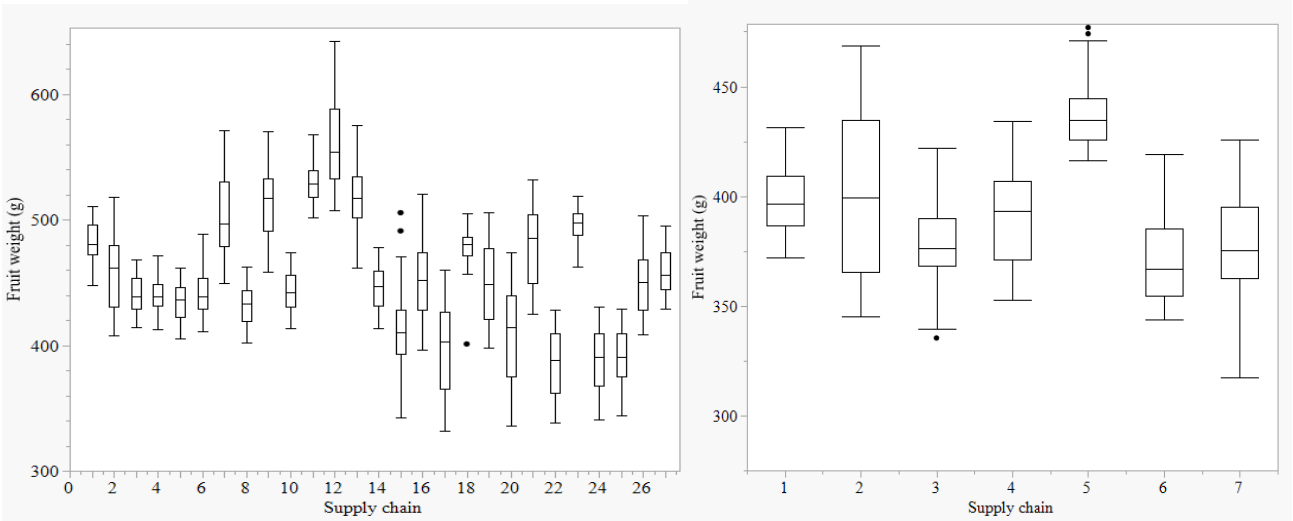
Gypsum	Ca: 23 % and S: 18%	1	Apr	1.5 kg / tree	Ca: 345 g
Calcium thiosulphate	Ca: 7.5% and S: 12.4%	4 (fertigation)	Jul - Oct	200 litres / ha	Ca: 42 ml
Boric acid	B: 16.5%	2	Jul and Sep		-
Zinc sulphate	Zn: 33%	2	Aug and Oct		-
Total N and Ca applied/tree					N: 384 and Ca: 387

Mg: Magnesium, Zn: Zinc, Mn: Manganese, S: Sulphur, Cu: Copper, Fe: Iron, B: Boron, Mo: Molybdenum, N: Nitrogen, P: Phosphorus, K: Potassium and Ca: Calcium.

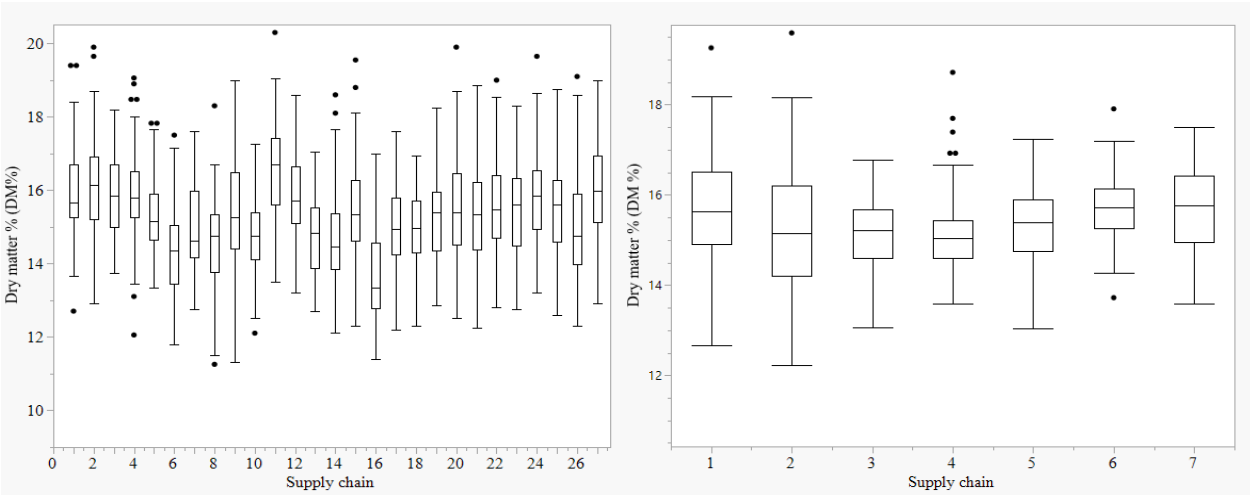
Appendix 12: Soil test parameters of 'B74' growing blocks registered for export. Export of 'B74' mangoes come from only two growers in Northern Territory (NT) and in North Queensland (NQ), respectively. Only data from three registered blocks from each site are tabulated. They were collected in 2022.

		Region					
		NT			NQ		
Soil test parameters	Optimal range	M38-2	M38-3	M56-1	M 36-4	M 36-1	M 36-3
pH	6 - 7	8.9	8.73	8.74	6.2	6.1	6.27
Potassium (ppm)	195 - 320	126	24.4	32.1	60.7	62.2	51.6
Calcium (ppm)	1300 - 2200	2106	1249	912	554	484	474
Zinc (ppm)	1.6 - 8	0.6	16.17	5.22	19.45	20.30	25.98
Boron (ppm)	1.7 - 4	0.4	0.42	0.47	0.31	0.28	0.19
Available N (kg / ha)	-	-	-	-	2.6	3.46	1.8
CEC (meq / 100g)	-	-	8.5	6.6	3.5	3.3	2.9
Base saturation (%)	80 - 87	-	100	100	-	-	-

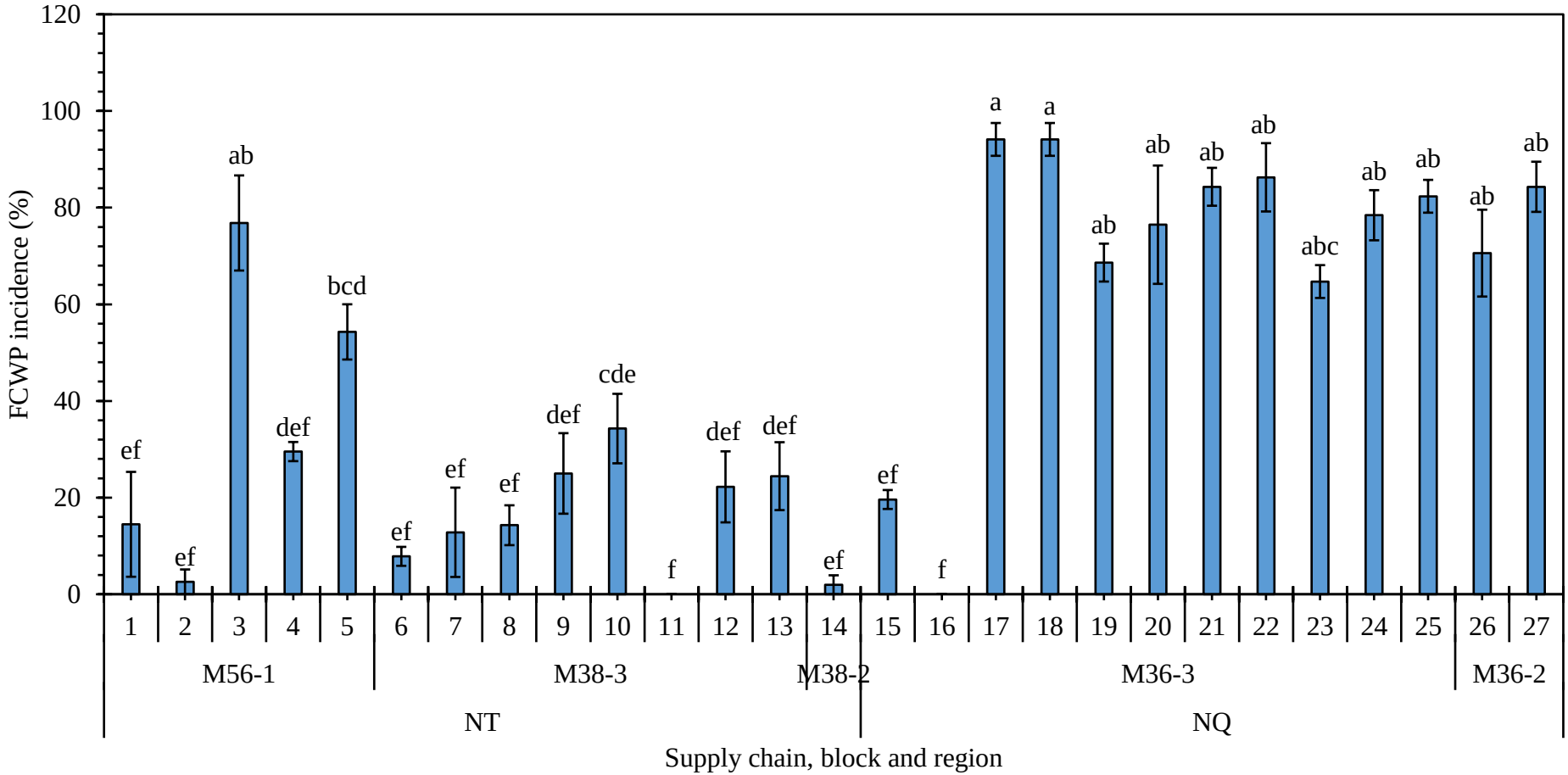
Appendix 13: ‘B74’ mango fruit weight distribution in supply chains covering two regions of Australia i.e., Northern Territory (NT) and North Queensland (NQ) in 2021/22 (left) and 2022/23 (right) harvest seasons. Dots represent outliers. Ends of lower and upper whiskers represent minimum and maximum data values, respectively. Lower whiskers represent 25% data set. Upper whisker represents the 25% data set. Boxes represent the intermediate 50% data set. Transverse line within the boxes is the median. Diagram was constructed in JMP Pro 16.0.0.



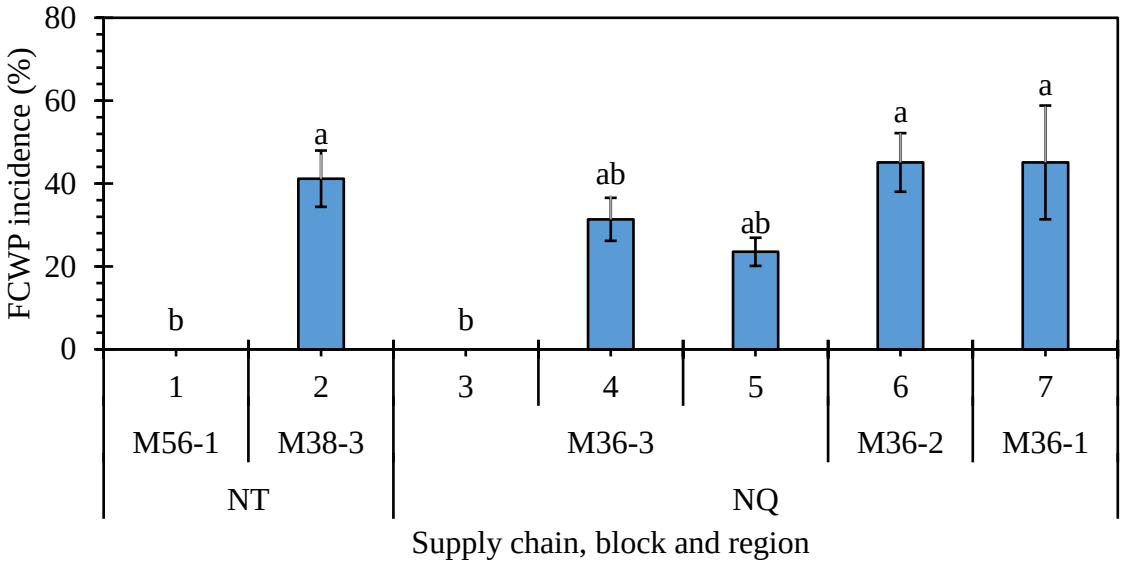
Appendix 14: ‘B74’ mango fruit dry matter percentage (DM%) distribution in supply chains covering two regions of Australia i.e., Northern Territory (NT) and North Queensland (NQ) in 2021/22 (left) and 2022/23 (right) harvest seasons. Dots represent outliers. Ends of lower and upper whiskers represent minimum and maximum data values, respectively. Lower whiskers represent 25% data set. Upper whisker represents the 25% data set. Boxes represent the intermediate 50% data set. Transverse line within the boxes is the median. Diagram was constructed in JMP Pro 16.0.0.



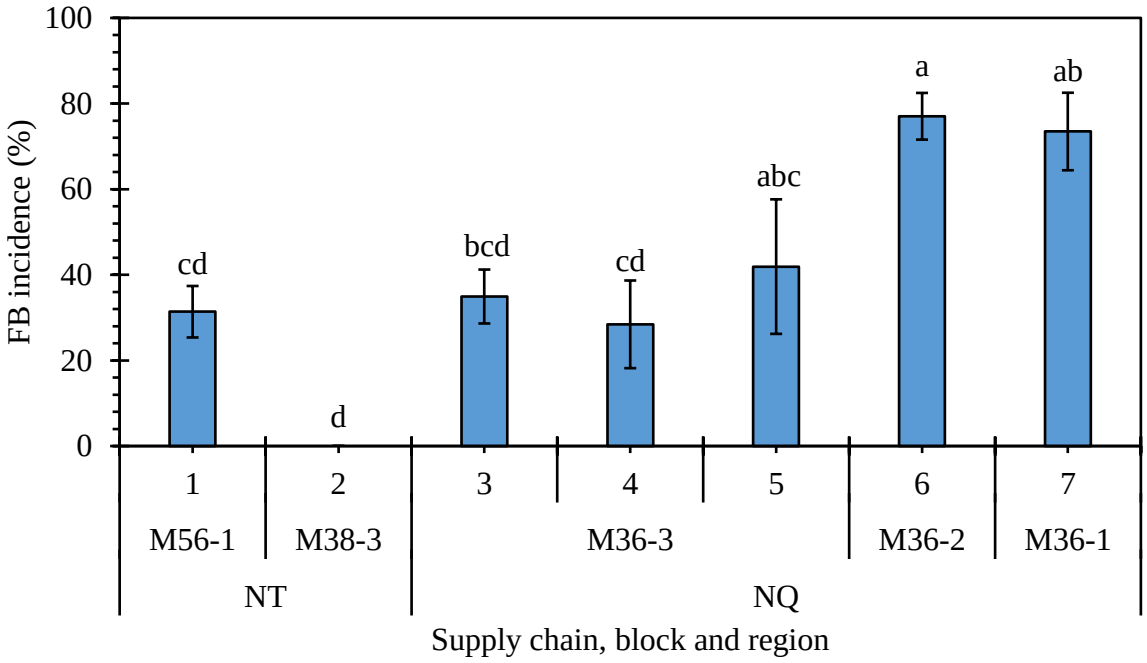
Appendix 15: Incidence and severity of flesh cavity with white patches (FCWP) in twenty-seven separate supply chains during the 2021/22 harvest season with vapour heat treatment (VHT). The data were collected from two regions in Australia, Northern Territory (NT) and North Queensland (NQ) with different harvest blocks from each growing region. Levels not connected by same letter are significantly different at 5% level of significance. Vertical bars represent ± standard error (SE) of means.



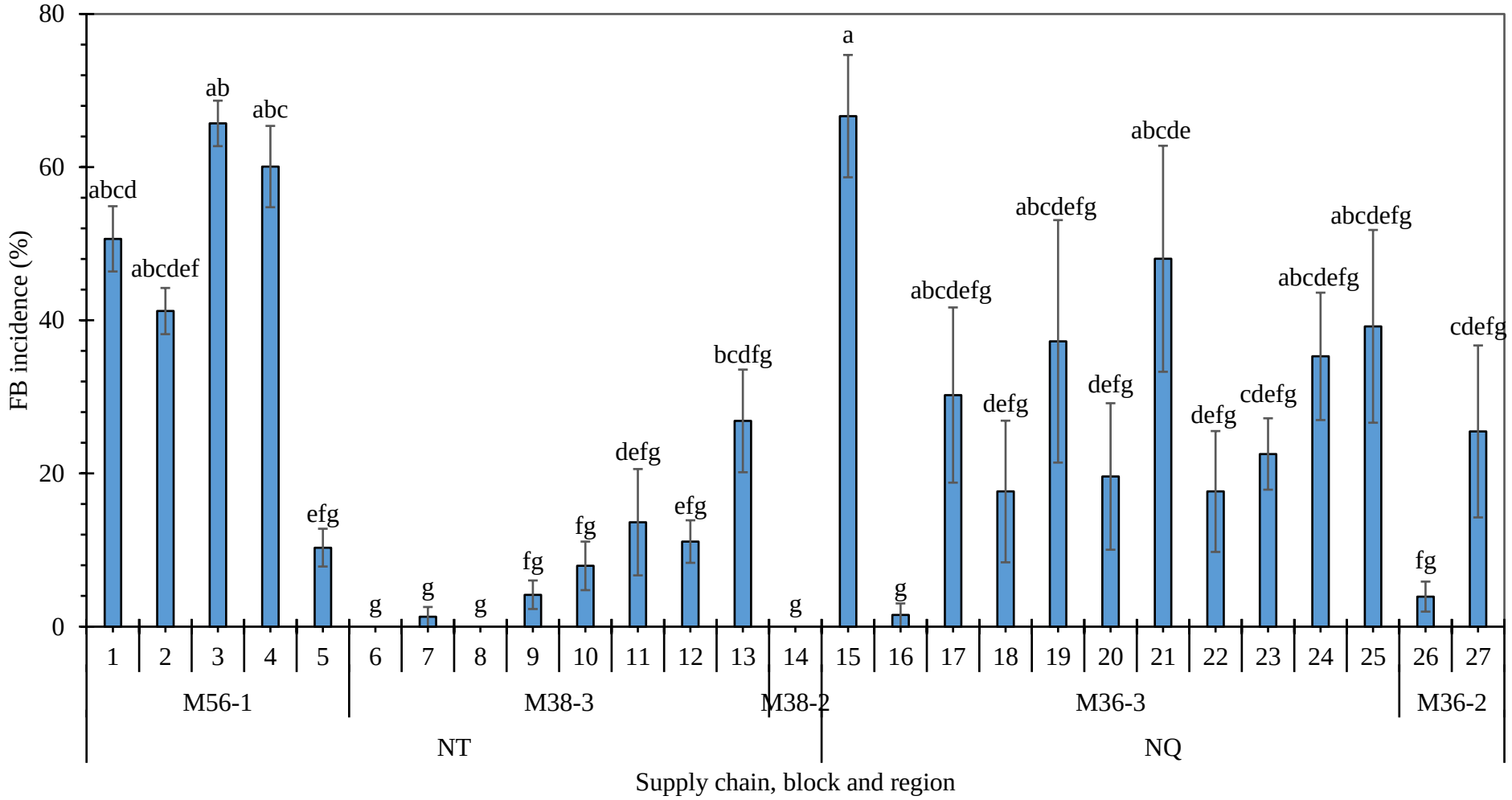
Appendix 16: Incidence of flesh cavity with white patches (FCWP) in seven separate supply chains during 2022/23 harvest season with vapour heat treatment (VHT). The data were collected from two regions in Australia, Northern Territory (NT) and North Queensland (NQ) with different harvest blocks from each region. Levels not connected by same letter are significantly different at 5% level of significance. Vertical bars represent ± standard error (SE) of means.



Appendix 17: Flesh browning (FB) incidence in seven separate case study supply chains during 2022/23 harvest season. The data were collected from two regions in Australia, Northern Territory (NT) and North Queensland (NQ) with different harvest blocks from each region. Levels not connected by same letter are significantly different at 5% level of significance. Vertical bars represent ± standard error (SE) of means.



Appendix 18: Flesh browning (FB) incidence in twenty-seven separate case study supply chains during the 2021/22 harvest season. The data were collected from two regions in Australia, Northern Territory (NT) and North Queensland (NQ) with different harvest blocks from each growing region. Levels not connected by same letter are significantly different at 5% level of significance. Vertical bars represent ± standard error (SE) of means.



Appendix 19: Effect of +/- vapour heat treatment (VHT) on shelf-life (mean ± SE) of 'B74' mango in twenty-seven case study supply chains during the 2021/22 harvest season. Shelf-life was calculated from the day of VHT to the fruit reaching hand firmness rating scale of 4. No data in supply chain 16 as only VHT fruit was available during monitoring.

Supply chain	Shelf-life (days)		
	+VHT	-VHT	Sig.
1	13.46 ± 0.83[b]	18.27 ± 0.13[a]	*
2	13.75 ± 0.25[b]	16.40 ± 0.82[a]	*
3	14.55 ± 1.41[b]	19.43 ± 0.97a	*
4	12.00 ± 1.73[b]	18.33 ± 0.33[a]	*
5	18.50 ± 0.32[b]	21.67 ± 0.43[a]	**
6	15.80 ± 0.71[b]	19.47 ± 0.61[a]	**
7	13.87 ± 0.99[b]	19.16 ± 0.73[a]	**
8	16.47 ± 0.92	16.94 ± 3.38	NS
9	15.64 ± 1.52	17.22 ± 2.42	NS
10	19.9 ± 0.45	21.13 ± 0.34	NS
11	21.98 ± 0.51[a]	20.61 ± 0.63[b]	*
12	19.75 ± 0.11	20.92 ± 0.92	NS
13	22.39 ± 0.84	23.3 ± 0.49	NS
14	16.35 ± 16.35	14.25 ± 0.67	NS
15	22.65 ± 0.07	22.88 ± 0.06	NS
16	-	-	-
17	18.08 ± 0.13[b]	19.47 ± 0.35[a]	*
18	20.16 ± 0.83	22.25 ± 0.62	NS
19	20.12 ± 1.09	21.44 ± 0.61	NS
20	20.51 ± 0.91[b]	23.88 ± 0.67[a]	*
21	20.87 ± 1.82	23.10 ± 0.56	NS
22	21.16 ± 0.42	22.18 ± 0.66	NS
23	18.7 ± 0.21[b]	24.08 ± 0.50[a]	**
24	22.33 ± 0.90	23.9 ± 1.21	NS
25	20.12 ± 0.89	21.79 ± 1.35	NS
26	21.76 ± 1.10	21.80 ± 0.53	NS
27	17.1 ± 0.99	23.02 ± 2.60	NS

VHT: Vapour heat treated; -VHT: Not vapour heat treated. Means followed by same letter in a row within incidence and severity are not statistically different. Significant codes *: 0.05, **: 0.01; ***: 0.001; NS: non-significant.

Appendix 20: Effect of +/- vapour heat treatment (VHT) on shelf-life (mean ± SE) of 'B74' mango in seven case study supply chains during the 2022/23 harvest season. Shelf-life was calculated from the day of VHT to the fruit reaching hand firmness rating scale of 4.

Supply chain	Shelf-life (days)		
	+VHT	-VHT	Sig.
1	19.65 ± 0.31	19.24 ± 0.57	NS
2	23.02 ± 1.00	21.53 ± 0.75	NS
3	18.61 ± 0.63	19.07 ± 0.49	NS
4	15.65 ± 0.94[b]	19.18 ± 0.69[a]	*
5	17.88 ± 1.50	20.15 ± 0.80	NS
6	22.16 ± 0.11	22.87 ± 0.36	NS
7	20.47 ± 0.49	22.88 ± 0.88	NS

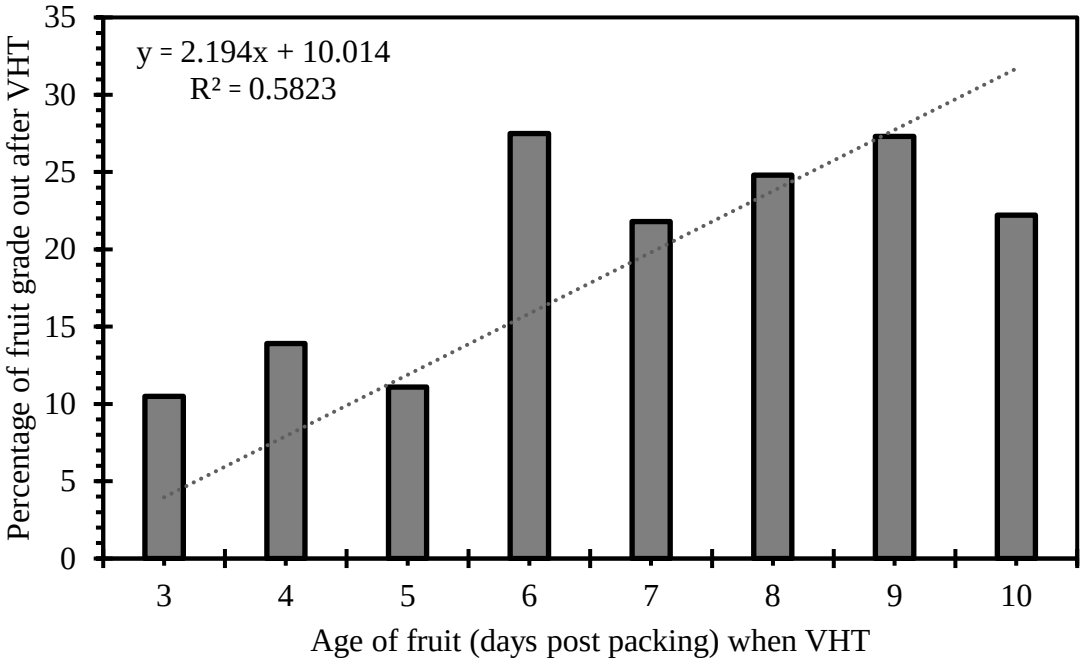
VHT: Vapour heat treated; -VHT: Not vapour heat treated. Means followed by same letter in a row within incidence and severity are not statistically different. Significant codes *: 0.05, **: 0.01; ***: 0.001; NS: non-significant.

Appendix 21: Incidence and severity of flesh cavity with white patches (FCWP) (mean ± SE) in seven separate case study supply chains during the 2022/23 harvest season with +/- vapour heat treatment (VHT). The data were collected from two regions in Australia, Northern Territory (NT; supply chains 1 and 2) and North Queensland (NQ; supply chains 3 - 7).

Supply chain	FCWP incidence			FCWP severity		
	+VHT	-VHT	Sig.	+VHT	-VHT	Sig.
1	0.00 ± 0.00	0.00 ± 0.00	NS	0.00 ± 0.00	0.00 ± 0.00	NS
2	41.18 ± 6.79[a]	0.00 ± 0.00[b]	*	0.56 ± 0.15[a]	0.00 ± 0.00[b]	*
3	0.00 ± 0.00	0.00 ± 0.00	NS	0.00 ± 0.00	0.00 ± 0.00	NS
4	31.37 ± 5.19[a]	0.00 ± 0.00[b]	*	0.33 ± 0.05[a]	0.00 ± 0.00[b]	*
5	23.53 ± 3.40[a]	0.00 ± 0.00[b]	*	0.23 ± 0.04[a]	0.00 ± 0.00[b]	*
6	45.1 ± 7.07[a]	0.00 ± 0.00[b]	*	0.50 ± 0.14[a]	0.00 ± 0.00[b]	*
7	45.1 ± 13.73[a]	0.00 ± 0.00[b]	*	0.56 ± 0.16[a]	0.00 ± 0.00[b]	*

+VHT: Vapour heat treated; -VHT: Not vapour heat treated. Means followed by same letter in a row within incidence and severity are not statistically different. Sig.: Significance; Significant codes *: 0.05, **: 0.01; ***: 0.001; NS: non-significant.

Appendix 22: The relationship between time from harvest to vapour heat treatment (VHT) and the percentage of grade-out caused by apparent skin damage in 'B74' mango fruit following VHT during the 2018/19 harvest season.



Appendix 23: Incidence and severity of flesh browning (FB) (mean ± SE) in nine separate case study supply chains during the 2020/21 harvest season with +/- vapour heat treatment (VHT). The data were collected from two regions in Australia, Northern Territory (NT; supply chains 1 - 5) and North Queensland (NQ; supply chains 6 - 9).

Supply chain	FB incidence			FB severity		
	+VHT	-VHT	Sig.	+VHT	-VHT	Sig.
1	21.11 ± 4.40	9.52 ± 4.76	NS	0.26 ± 0.06	0.09 ± 0.05	NS
2	33.95 ± 3.65	22.83 ± 6.83	NS	0.39 ± 0.08	0.40 ± 0.08	NS
3	27.94 ± 10.29	35.42 ± 15.45	NS	0.27 ± 0.13	0.46 ± 0.16	NS
4	21.43 ± 8.05	25.46 ± 6.71	NS	0.25 ± 0.09	0.36 ± 0.14	NS
5	12.13 ± 3.72	21.81 ± 4.96	NS	0.15 ± 0.04	0.29 ± 0.06	NS
6	4.04 ± 2.02	0.00 ± 0.00	NS	0.06 ± 0.04	0.00 ± 0.00	NS
7	4.55 ± 4.55	0.00 ± 0.00	NS	0.06 ± 0.06	0.00 ± 0.00	NS
8	1.96 ± 1.96	6.00 ± 0.12	NS	0.02 ± 0.02	0.06 ± 0.00	NS
9	1.96 ± 1.96	0.00 ± 0.00	NS	0.04 ± 0.04	0.00 ± 0.00	NS

+VHT: Vapour heat treated; -VHT: Not vapour heat treated. Means followed by same letter in a row within incidence and severity are not statistically different. NS: non-significant.

Appendix 24: Incidence and severity of flesh browning (FB) (mean ± SE) in twenty-seven separate supply chains during 2021/22 harvest season with +/- vapour heat treatment (VHT). The data were collected from two regions in Australia, Northern Territory (NT; supply chains 1 - 14) and North Queensland (NQ; supply chains 15 - 27).

Supply chain	FB incidence			FB severity		
	+VHT	-VHT	Sig.	+VHT	-VHT	Sig.
1	44.87 ± 3.39	56.41 ± 6.78	NS	0.53 ± 0.07	0.59 ± 0.08	NS
2	39.74 ± 5.59	42.67 ± 3.48	NS	0.51 ± 0.03	0.44 ± 0.12	NS
3	63.49 ± 3.17	67.94 ± 5.40	NS	0.62 ± 0.16^{b}	1.14 ± 0.05^{a}	*
4	49.84 ± 5.20^{b}	70.32 ± 3.02^{a}	*	0.67 ± 0.08	0.92 ± 0.11	NS
5	9.05 ± 2.15	11.59 ± 4.92	NS	0.07 ± 0.06	0.10 ± 0.00	NS
6	0.00 ± 0.00	0.00 ± 0.00	NS	0.00 ± 0.00	0.00 ± 0.00	NS
7	0.00 ± 0.00	2.56 ± 2.56	NS	0.00 ± 0.03	0.03 ± 0.00	NS
8	0.00 ± 0.00	0.00 ± 0.00	NS	0.00 ± 0.00	0.00 ± 0.00	NS
9	5.56 ± 2.78	2.78 ± 2.78	NS	0.06 ± 0.03	0.03 ± 0.03	NS
10	2.38 ± 2.38	13.49 ± 3.71	NS	0.02 ± 0.03	0.09 ± 0.02	NS
11	6.06 ± 3.03	21.21 ± 13.21	NS	0.03 ± 0.16	0.23 ± 0.02	NS
12	8.33 ± 4.81	13.89 ± 2.78	NS	0.08 ± 0.03	0.11 ± 0.05	NS
13	33.02 ± 3.23	20.71 ± 13.28	NS	0.33 ± 0.17	0.27 ± 0.03	NS
14	0.00 ± 0.00	0.00 ± 0.00	NS	0.00 ± 0.00	0.00 ± 0.00	NS
15	49.02 ± 1.96^{b}	84.31 ± 1.96^{a}	***	0.45 ± 0.02^{b}	0.90 ± 0.05^{a}	**
16	3.03 ± 3.03	-	-	0.02 ± 0.00	-	-
17	5.88 ± 0.00^{b}	54.60 ± 7.80^{a}	*	0.05 ± 0.08	0.49 ± 0.01^{a}	*
18	1.96 ± 1.96	33.33 ± 13.33	NS	0.02 ± 0.11^{b}	0.34 ± 0.02^{a}	*
19	1.96 ± 1.96^{b}	72.55 ±1.96^{a}	***	0.01 ± 0.08^{b}	0.99 ± 0.01^{a}	**
20	0.00 ± 0.00^{b}	39.22 ± 8.55^{a}	*	0.00 ± 0.18	0.46 ± 0.00	NS
21	15.69 ± 3.92^{b}	80.39 ± 5.19^{a}	***	0.17 ± 0.22^{b}	1.77 ± 0.03^{a}	**
22	5.88 ± 5.88	29.41 ± 11.76	NS	0.09 ± 0.16	0.26 ± 0.09	NS
23	15.69 ± 7.07	29.41 ± 3.40	NS	0.17 ± 0.04	0.34 ± 0.09	NS
24	19.61 ± 7.07^{b}	50.98 ± 7.07^{a}	*	0.20 ± 0.09^{b}	0.55 ± 0.07^{a}	*
25	11.76 ± 5.88^{b}	66.67 ± 1.96^{a}	**	0.15 ± 0.15^{b}	1.16 ± 0.08^{a}	**
26	0.00 ± 0.00^{b}	7.84 ± 1.96^{a}	*	0.00 ± 0.03	0.06 ± 0.00	NS

27	1.96 ± 1.96[b]	49.02 ± 8.55[a]	*	0.01 ± 0.12[b]	0.47 ± 0.01[a]	*

+VHT: Vapour heat treated; -VHT: Not vapour heat treated. Means followed by same letter in a row within incidence and severity are not statistically different. Significant codes *: 0.05, **: 0.01; ***: 0.001; NS: non-significant.

Appendix 25: Incidence and severity of flesh browning (FB) (mean ± SE) in seven separate case study supply chains during the 2022/23 harvest season with +/- vapour heat treatment (VHT). The data were collected from two regions in Australia, Northern Territory (NT, supply chains 1and 2) and North Queensland (NQ, supply chains 3 - 7).

Supply	FB incidence			FB severity		
chain	+VHT	-VHT	Sig.	+VHT	-VHT	Sig.
1	39.22 ± 10.38	23.53 ± 3.40	NS	0.75 ± 0.12	0.51 ± 0.14	NS
2	0.00 ± 0.00	0.00 ± 0.00	NS	0.00 ± 0.00	0.00 ± 0.00	NS
3	25.49 ± 5.19	44.36 ± 9.07	NS	0.25 ± 0.09	0.39 ± 0.05	NS
4	50.98 ± 1.96[b]	5.88 ± 3.40[a]	***	0.08 ± 0.02[b]	0.84 ± 0.01[a]	***
5	7.84 ± 5.19b	75.98 ± 6.81a	**	0.09 ± 0.06a	1.44 ± 0.15b	**
6	78.43 ± 5.19a	75.61 ± 10.94	*	0.82 ± 0.12	1.09 ± 0.08	**
7	54.90±7.84	92.03 ± 1.90	*	0.63 ± 0.07	1.48 ± 0.08	**

VHT: Vapour heat treated; -VHT: Not vapour heat treated. Means followed by same letter in a row within incidence and severity are not statistically different. Significant codes *: 0.05, **: 0.01; ***: 0.001; NS: non-significant.

Appendix 26: Analysis of variance (ANOVA) of flesh cavity with white patches (FCWP) severity in fruit of different maturity, +/-VHT and their interactions 2022 harvest season.

Source	df	Sum of squares	F ratio	Prob > F
Maturity	2	0.10	4.14	0.0428*
Treatments	1	0.12	10.52	0.0072*
Maturity × treatments	2	0.10	4.14	0.0428*

Appendix 27: Analysis of variance (ANOVA) of flesh cavity with white patches (FCWP) incidence in fruit of different maturity, +/-vapour heat treatment (VHT) and their interactions 2022 harvest season.

Source	df	Sum of squares	F ratio	Prob > F
Maturity	2	353.00	7.31	0.0084*
Treatments	1	695.89	28.81	0.0002*

Maturity × treatments	2	353.00	7.31	0.0084*

Appendix 28: Analysis of variance (ANOVA) of flesh cavity with white patches (FCWP) severity in fruit harvested at different maturity stages with and without conditioning prior to vapour heat treatment (VHT) and their interactions in 2022 harvest season.

Source	df	Sum of squares	F ratio	Prob > F
Maturity	2	0.23	17.76	0.0012*
Conditioning	2	0.88	33.71	<0.0001*
Maturity × conditioning	5	0.36	13.80	0.0008*

Appendix 29: Analysis of variance (ANOVA) of flesh cavity with white patches (FCWP) incidence in fruit harvested at different maturity stages with and without conditioning prior to vapour heat treatment and their interactions in 2022 harvest season.

Source	df	Sum of squares	F ratio	Prob > F
Maturity	2	1547.00	26.03	0.0003*
Conditioning	2	6937.96	58.37	<0.0001*
Maturity × conditioning	5	2250.44	18.93	0.0002*

Appendix 30: Analysis of variance (ANOVA) of flesh browning (FB) severity in fruit harvested at different maturity stages with and without conditioning prior to vapour heat treatment (VHT) and their interactions in 2022 harvest season.

Source	df	Sum of squares	F ratio	Prob > F
Maturity	2	0.00	3	0.11
Conditioning	2	0.00	0.00	1.00
Maturity × conditioning	5	0.00	0.00	1.00

Appendix 31: Analysis of variance (ANOVA) of flesh browning (FB) incidence in fruit harvested at different maturity stages with and without conditioning prior to vapour heat treatment and their interactions in 2022 harvest season.

Source	df	Sum of squares	F ratio	Prob > F
Maturity	2	34.72	2.78	0.12
Conditioning	2	2.78	0.11	0.90
Maturity × conditioning	5	2.78	0.11	0.90

Appendix 32: Analysis of variance (ANOVA) of flesh browning (FB) severity in fruit of different maturity sorted by floatation technique, storage conditions and their interactions in 2022 harvest season.

Source	df	Sum of squares	F ratio	Prob > F
Maturity	1	0.08	2.94	0.1247
Storage	1	0.82	28.88	0.0007*
Maturity × storage	1	0.04	1.31	0.286

Appendix 33: Analysis of variance (ANOVA) of flesh browning (FB) incidence in fruit of different maturity sorted by floatation technique, storage conditions and their interactions in 2022 harvest season.

Source	df	Sum of squares	F ratio	Prob > F
Maturity	1	370.37	1.85	0.2107
Storage	1	4281.48	21.41	0.0017
Maturity × storage	1	133.33	0.67	0.4379

Appendix 34: Analysis of variance (ANOVA) of flesh browning (FB) severity in fruit of different maturity sorted by floatation technique, storage conditions and their interactions in 2023 harvest season.

Source	df	Sum of squares	F ratio	Prob > F
Maturity	1	0.01	0.16	0.6953
Storage	1	1.16	32.33	0.0005*
Maturity × storage	1	0.01	0.26	0.6254

Appendix 35: Analysis of variance (ANOVA) of flesh browning (FB) incidence in fruit of different maturity sorted by floatation technique, storage conditions and their interactions in 2023 harvest season.

Source	df	Sum of squares	F ratio	Prob > F
Maturity	1	0.00	0.00	1.00
Storage	1	5348.15	48.13	0.0001*
Maturity × storage	1	59.26	0.53	0.4860

Appendix 36: Analysis of variance (ANOVA) of CO_2 production on day 1 among the different mature fruit (less mature: <15% DMC, optimum mature: 15 - 17% DMC and more mature: >17% DMC) +/- VHT in 2023 harvest season.

Source	df	Sum of squares	Mean square	F ratio	Prob > F
Treatment	5	870.79	174.16	4.19	0.0196*
Error	12	498.96	41.58		
C. Total	17	1369.60			

Appendix 37: Analysis of variance (ANOVA) of CO_2 production on day 2 among the different mature fruit (less mature: <15% DMC, optimum mature: 15 - 17% DMC and more mature: >17% DMC) +/- VHT in 2023 harvest season.

Source	df	Sum of squares	Mean square	F ratio	Prob > F
Treatment	5	898.92	179.78	3.35	0.0399*
Error	12	643.39	53.62		
C. Total	17	1542.32			

Appendix 38: Analysis of variance (ANOVA) of CO_2 production on day 3 among the different mature fruit (less mature: <15% DMC, optimum mature: 15 - 17% DMC and more mature: >17% DMC) +/- VHT in 2023 harvest season.

Source	df	Sum of squares	Mean square	F ratio	Prob > F
Treatment	5	981.95	196.39	3.36	0.0397*
Error	12	701.96	58.50		
C. Total	17	1683.91			

Appendix 39: Mean separation of CO2 production on day 1, 2 and 3 among different mature fruit (less mature: <15% DMC, optimum mature: 15 - 17% DMC and more mature: >17% DMC) +/- VHT in 2023 harvest season.

	Average CO_2 production (mg/kg/hr)		
Treatment	Day 1	Day 2	Day 3
Less mature non-VHT	20.00b	25.39b	28.78b
Less mature VHT	30.78ab	33.91ab	39.77ab
Optimum mature non-VHT	29.06ab	37.33ab	41.11ab
Optimum mature VHT	41.42a	47.08a	49.57ab
More mature non-VHT	27.86ab	37.37ab	37.62ab
More mature VHT	37.87a	44.46ab	50.58a

Appendix 40: Analysis of variance to assess the effect of maturity (less mature: <15% DMC, optimum mature: 15-17% DMC and more mature: >17% DMC) and treatment (with and without vapour heat treatment (VHT)) on spatial (inner, middle, and outer mesocarp) and temporal (day after VHT to end of shelf-life i.e., 12 days) changes on T2 value of 'B74' mango fruit mesocarp.

Source	Day 0	Day 3	Day 6	Day 9	Day 12
Mesocarp position	<.0001*	<.0001*	<.0001*	0.0003*	0.0814
Maturity	0.0281*	0.0058*	0.3936	0.6810	0.5021
Mesocarp position × Maturity	0.1317	0.1308	0.1145	0.0296*	0.0176*
Treatment	0.0057*	0.0030*	<.0001*	0.0006*	0.0033*
Mesocarp position × Treatment	0.0796	<.0001*	0.0006*	0.0033*	0.0212*
Maturity × Treatment	0.3122	0.0962	0.2231	0.7247	0.8205
Mesocarp position × Maturity × Treatment	0.9652	0.4335	0.8518	0.8981	0.4697

Appendix 41: Analysis of variance to assess the effect of maturity (less mature: <15% DMC, optimum mature: 15-17% DMC and more mature: >17% DMC) and treatment (with and without vapour heat treatment (VHT)) on spatial (inner, middle, and outer mesocarp) and temporal (day after VHT to end of shelf-life i.e., 12 days) changes in PD value of 'B74' mango fruit mesocarp.

Source	Day 0	Day 3	Day 6	Day 9	Day 12
Mesocarp position	<.0001*	0.0005*	<.0001*	0.0002*	0.0315*
Maturity	<.0001*	<.0001*	<.0001*	0.0022*	0.0534
Mesocarp position × Maturity	0.8719	0.5896	0.9255	0.9814	0.3526
Treatment	0.0366*	<.0001*	0.1446	0.5809	0.4810
Mesocarp position × Treatment	0.7187	0.3641	0.8291	0.6738	0.4682
Maturity × Treatment	<.0001*	0.3020	0.0006*	0.0014*	0.0009*
Mesocarp position × Maturity × Treatment	0.9713	0.8218	0.9886	0.9986	0.8670

Appendix 42: Analysis of variance to assess the effect of maturity (less mature: <15% DMC, optimum mature: 15 - 17% DMC and more mature: >17% DMC) and treatment (with and without vapour heat treatment (VHT)) on fruit firmness of 'B74' mango fruit at different stages of ripening (day after VHT to end of shelf-life i.e., 12 days).

Source	Day 0	Day 3	Day 6	Day 9	Day 12
Maturity	-	0.1007	0.0707	0.0104	0.0079
Treatment	-	<0.0001	0.0003	0.0004	0.0005*
Maturity × treatment	-	0.7602	0.6719	0.7019	0.3814

Appendix 43: Analysis of variance to assess the effect of maturity (less mature: <15% DMC, optimum mature: 15 - 17% DMC and more mature: >17% DMC) and treatment (with and without vapour heat treatment (VHT)) on TSS of 'B74' mango fruit at different stages of ripening (day after VHT to end of shelf-life i.e., 12 days).

Source	Day 0	Day 3	Day 6	Day 9	Day 12
Maturity	0.0646	0.0096*	0.0315*	0.0089*	0.0959
Treatment	0.3380	0.0814	0.1015	0.0984	0.0021*
Maturity × treatment	0.9933	0.3416	0.2919	0.6380	0.0284*

Appendix 44: Analysis of variance to assess the spatial (inner, middle, and outer mesocarp) variation of mesocarp porosity.

Source	df	Sum of Squares	Mean Square	F Ratio	Prob > F
Position	2	39162391	19581196	10.7679	0.0003*
Error	33	60009731	1818476.7		
C. Total	35	99172122			

Appendix 45: Temperature increase pattern inside fruit during vapour heat treatment process.

20.11.29 at 09-25-01 Treat.VHT
BATCH NUMBER : 20049

Logging Started Sunday, 29 November 2020 09:25 AM

	%RH	DB	WB	01	02	03	04	05	06	07	08	09	10
				11	12	13	14	15	16	17	18	19	20
09:25 AM	87.9	23.9	22.4	x	x	x	x	x	x	x	x	x	x
				18.9	18.8	19.8	20.2	19.3	18.7	19.6	19.5	20.0	x
09:30 AM	69.6	30.2	25.6	x	x	x	x	x	x	x	x	x	x
				19.2	19.3	20.1	20.5	19.6	19.1	20.1	19.8	20.4	x
09:35 AM	59.5	34.5	27.6	x	x	x	x	x	x	x	x	x	x
				19.8	20.1	20.6	21.1	20.0	19.7	21.0	20.3	21.1	x
09:40 AM	54.3	37.3	29.0	x	x	x	x	x	x	x	x	x	x
				20.4	21.0	21.4	22.0	20.6	20.4	22.0	20.9	21.9	x
09:45 AM	51.5	39.3	30.1	x	x	x	x	x	x	x	x	x	x
				21.2	21.9	22.3	22.8	21.3	21.3	23.1	21.7	22.8	x
09:50 AM	49.7	41.1	31.1	x	x	x	x	x	x	x	x	x	x
				22.0	22.8	23.3	23.8	22.1	22.2	24.2	22.4	23.7	x
09:55 AM	48.4	42.6	32.1	x	x	x	x	x	x	x	x	x	x
				22.9	23.8	24.3	24.7	23.0	23.2	25.4	23.2	24.7	x
10:00 AM	47.4	43.9	32.9	x	x	x	x	x	x	x	x	x	x
				23.8	24.7	25.4	25.7	23.9	24.2	26.5	24.1	25.8	x
10:05 AM	47.9	44.5	33.5	x	x	x	x	x	x	x	x	x	x
				24.7	25.7	26.5	26.7	24.8	25.2	27.7	25.0	26.8	x
10:10 AM	47.0	45.8	34.3	x	x	x	x	x	x	x	x	x	x
				25.7	26.6	27.6	27.7	25.8	26.2	28.9	25.8	27.8	x
10:15 AM	58.5	44.4	36.1	x	x	x	x	x	x	x	x	x	x
				26.6	27.6	28.8	28.7	26.7	27.3	30.0	26.7	28.9	x
10:20 AM	70.7	43.9	38.3	x	x	x	x	x	x	x	x	x	x
				27.8	28.9	29.8	29.9	27.7	28.4	31.2	27.8	30.2	x
10:25 AM	73.0	44.1	39.1	x	x	x	x	x	x	x	x	x	x
				29.1	30.4	30.9	31.1	28.9	29.6	32.4	29.0	31.5	x
10:30 AM	74.2	44.7	39.8	x	x	x	x	x	x	x	x	x	x
				30.5	31.7	32.0	32.3	30.1	30.8	33.5	30.3	32.7	x
10:35 AM	75.2	45.4	40.7	x	x	x	x	x	x	x	x	x	x
				31.8	33.0	33.1	33.4	31.3	32.0	34.5	31.5	33.8	x
10:40 AM	75.7	46.1	41.5	x	x	x	x	x	x	x	x	x	x
				33.0	34.2	34.1	34.5	32.4	33.1	35.5	32.6	34.8	x
10:45 AM	76.0	46.9	42.2	x	x	x	x	x	x	x	x	x	x
				34.1	35.3	35.2	35.5	33.6	34.2	36.4	33.7	35.8	x
10:50 AM	76.1	47.7	43.0	x	x	x	x	x	x	x	x	x	x
				35.2	36.4	36.1	36.6	34.6	35.3	37.4	34.8	36.8	x
10:55 AM	76.4	48.3	43.7	x	x	x	x	x	x	x	x	x	x
				36.3	37.4	37.1	37.5	35.7	36.3	38.3	35.8	37.7	x
11:00 AM	77.4	48.8	44.3	x	x	x	x	x	x	x	x	x	x
				37.3	38.4	38.0	38.5	36.7	37.3	39.2	36.8	38.6	x
11:05 AM	78.3	49.1	44.9	x	x	x	x	x	x	x	x	x	x
				38.2	39.3	38.9	39.4	37.7	38.2	40.0	37.8	39.4	x
11:10 AM	80.1	49.2	45.3	x	x	x	x	x	x	x	x	x	x
				39.1	40.2	39.7	40.2	38.6	39.1	40.8	38.7	40.2	x
11:15 AM	81.6	49.2	45.6	x	x	x	x	x	x	x	x	x	x
				40.0	40.9	40.5	41.0	39.5	39.9	41.5	39.5	40.9	x
11:20 AM	83.0	49.2	45.9	x	x	x	x	x	x	x	x	x	x
				40.7	41.6	41.3	41.7	40.2	40.7	42.1	40.3	41.6	x
11:25 AM	84.5	49.1	46.1	x	x	x	x	x	x	x	x	x	x
				41.5	42.3	41.9	42.3	41.0	41.4	42.7	41.0	42.2	x
11:30 AM	85.9	49.1	46.4	x	x	x	x	x	x	x	x	x	x
				42.1	42.9	42.5	42.9	41.6	42.0	43.2	41.6	42.7	x
11:35 AM	87.0	49.1	46.6	x	x	x	x	x	x	x	x	x	x
				42.7	43.4	43.0	43.4	42.2	42.6	43.7	42.2	43.2	x
11:40 AM	88.2	49.1	46.8	x	x	x	x	x	x	x	x	x	x
				43.3	43.9	43.5	43.9	42.8	43.1	44.1	42.8	43.7	x
11:45 AM	89.2	49.1	47.0	x	x	x	x	x	x	x	x	x	x
				43.7	44.3	44.0	44.4	43.3	43.6	44.5	43.3	44.0	x
11:50 AM	90.2	49.0	47.2	x	x	x	x	x	x	x	x	x	x

RH90%

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20.11.29 at 09-25-01 Treat.VHT

				44.2	44.7	44.4	44.8	43.8	44.1	44.8	43.7	44.4	x
11:55 AM	90.7	49.1	47.4	x	x	x	x	x	x	x	x	x	x
				44.6	45.1	44.7	45.2	44.2	44.5	45.2	44.2	44.8	x
12:00 PM	91.6	49.1	47.5	x	x	x	x	x	x	x	x	x	x
				45.0	45.4	45.1	45.5	44.7	44.9	45.5	44.5	45.1	x
12:05 PM	92.3	49.1	47.6	x	x	x	x	x	x	x	x	x	x
				45.4	45.7	45.4	45.8	45.0	45.2	45.7	44.9	45.4	x
12:10 PM	93.3	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				45.7	46.0	45.7	46.1	45.3	45.5	46.0	45.2	45.6	x
12:15 PM	93.1	49.1	47.8	x	x	x	x	x	x	x	x	x	x
				46.0	46.3	46.0	46.4	45.6	45.8	46.2	45.5	45.9	x
12:20 PM	93.1	49.1	47.9	x	x	x	x	x	x	x	x	x	x
				46.2	46.5	46.2	46.6	45.9	46.1	46.4	45.7	46.1	x
12:25 PM	93.2	49.1	47.9	x	x	x	x	x	x	x	x	x	x
				46.5	46.7	46.4	46.8	46.1	46.3	46.5	45.9	46.2	x
12:30 PM	93.1	49.1	47.8	x	x	x	x	x	x	x	x	x	x
				46.6	46.8	46.6	47.0	46.3	46.5	46.7	46.1	46.3	x
12:35 PM	93.4	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				46.8	46.9	46.8	47.1	46.5	46.7	46.8	46.3	46.4	x
12:40 PM	93.4	49.1	47.8	x	x	x	x	x	x	x	x	x	x
				47.0	47.0	46.9	47.2	46.6	46.8	46.9	46.4	46.5	x
12:45 PM	93.5	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.1	47.1	47.1	47.4	46.8	47.0	47.0	46.5	46.6	x
12:50 PM	93.4	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.2	47.2	47.2	47.4	46.9	47.1	47.1	46.6	46.6	x
12:55 PM	93.4	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.3	47.2	47.2	47.5	47.0	47.2	47.1	46.6	46.7	x
01:00 PM	93.6	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.4	47.3	47.3	47.6	47.0	47.3	47.2	46.7	46.8	x
01:05 PM	93.6	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.5	47.3	47.4	47.7	47.1	47.4	47.3	46.8	46.9	x
01:10 PM	93.9	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.6	47.4	47.5	47.8	47.2	47.6	47.4	46.8	46.9	x
01:15 PM	93.4	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.7	47.4	47.6	47.9	47.2	47.6	47.5	46.9	47.0	x
01:17 PM	93.5	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.8	47.5	47.6	47.9	47.3	47.7	47.5	47.0	47.0	x
01:18 PM	93.5	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.8	47.5	47.6	48.0	47.3	47.7	47.6	47.0	47.1	x
01:19 PM	93.4	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.8	47.5	47.7	48.0	47.3	47.7	47.6	47.0	47.1	x
01:20 PM	93.5	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.8	47.5	47.7	48.0	47.3	47.8	47.6	47.0	47.1	x
01:21 PM	93.4	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.8	47.5	47.7	48.0	47.3	47.8	47.6	47.0	47.1	x
01:22 PM	93.5	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.9	47.5	47.7	48.0	47.3	47.8	47.6	47.0	47.1	x
01:23 PM	93.5	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.9	47.5	47.7	48.0	47.3	47.8	47.6	47.1	47.1	x
01:24 PM	93.4	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.9	47.5	47.7	48.1	47.4	47.8	47.6	47.1	47.2	x
01:25 PM	93.5	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.9	47.5	47.8	48.1	47.4	47.9	47.7	47.1	47.2	x
01:26 PM	93.4	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.9	47.5	47.8	48.1	47.4	47.9	47.7	47.1	47.2	x
01:27 PM	93.3	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.9	47.5	47.8	48.1	47.4	47.9	47.7	47.1	47.2	x
01:28 PM	93.4	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				47.9	47.6	47.8	48.1	47.4	47.9	47.7	47.1	47.2	x
01:29 PM	93.3	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				48.0	47.6	47.8	48.1	47.4	47.9	47.7	47.1	47.2	x
01:30 PM	93.5	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				48.0	47.6	47.8	48.2	47.4	47.9	47.7	47.2	47.2	x
01:31 PM	93.8	49.0	47.8	x	x	x	x	x	x	x	x	x	x
				48.0	47.6	47.9	48.2	47.4	48.0	47.8	47.2	47.3	x
01:32 PM	93.7	49.0	47.8	x	x	x	x	x	x	x	x	x	x

Treatment Start

D.D.Mahr

A03681

29/11/20

20.11.29 at 09-25-01 Treat.VHT

				48.0	47.6	47.9	48.2	47.4	48.0	47.8	47.2	47.3	x **
01:33 PM	92.7	48.9	47.5	x	x	x	x	x	x	x	x	x	x Treatment
				48.1	47.6	47.9	48.2	47.4	48.0	47.8	47.2	47.3	x **
01:38 PM	76.5	46.2	41.7	x	x	x	x	x	x	x	x	x	x Finish
				48.1	47.5	47.9	48.1	47.4	48.1	47.7	47.2	47.2	x **
01:43 PM	66.4	44.3	37.8	x	x	x	x	x	x	x	x	x	x P.D.Holmes
				42.6	43.5	39.2	45.1	44.4	45.0	44.2	44.9	44.4	x
01:48 PM	60.4	42.2	34.6	x	x	x	x	x	x	x	x	x	x A03681
				42.2	41.9	40.8	43.0	42.3	42.9	42.9	43.1	42.6	x
01:53 PM	60.0	40.8	33.3	x	x	x	x	x	x	x	x	x	x
				41.0	40.8	41.1	41.5	41.1	41.5	41.9	41.7	41.3	x
01:58 PM	59.8	39.6	32.1	x	x	x	x	x	x	x	x	x	x
				40.0	39.9	40.2	40.4	40.3	40.6	41.1	40.9	40.6	x

Logging Stopped Sunday, 29 November 2020 02:01 PM

Appendix 46: Confusion matrix of internal disorders flesh cavity with white patches and flesh browning by in-line NIR scanning.

Confusion Matrix for White Patches

Actual Grade \ Predicted Grade	0	1	2	3
0	257	421	6	0
1	61	427	32	0
2	21	213	73	0
3	3	50	26	0

Confusion Matrix for Flesh Browning

Actual Grade \ Predicted Grade	0	1	2	3
0	1419	2	0	0
1	131	1	0	0
2	27	0	0	0
3	10	0	0	0

Appendix 47: ‘B74’ mango fruit market specifications (https://bgbrisbane.com.au/wp-content/uploads/2016/12/Mango.pdf.) and the USDA quality check protocol (https: //efaidnbmnnnibpcajpcglclefindmkaj/https://www.ams.usda.gov/sites/default/files/media/Mango_Inspection_Instructions%5B1%5D.pdf.).

Market Produce Specifications

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PRODUCE: MANGO
TYPE: Ripened
VARIETY: Zango, B74, Calypso
CLASS: One

GENERAL APPEARANCE CRITERIA	
COLOUR	*Pale yellow with or without a pink to red blush; None with >40% of visible surface with light green colour; no chimeral variants (discoloured lines) with >1 line of green, not >2mm wide or 5cm long; not sunken or raised.*
VISUAL APPEARANCE	*Yellow flesh. With need for stickers with PLU and produce/variety name, or bar code when available, per requirements.*
SENSORY	*Firm, yields slightly to finger pressure; smooth skin; sweet, with some acid; pleasant aroma; no unpleasant odours/flavours (abnormal ripening).*
SHAPE	*Round to oval heart shaped. 'Flower' like growth around stem end is allowable, nil with excessively protruding areas.*
SIZE	*In pre-ordered size per requirements; uniform per tray.*
MATURITY	*Fully coloured ripened fruit.*
MAJOR DEFECTS	
INSECTS	*With evidence of live insects.*
DISEASES	*With fungal or bacterial rots (decaying areas).*
PHYSICAL / PEST DAMAGE	*With cuts, holes or cracks (that break the skin).*
TEMPERATURE INJURY	*With scattered, small dark-brown spotting on dull, discoloured skin. (chilling injury)*
PHYSIOLOGICAL DISORDERS	*With internal breakdown, eg. watery, translucent area in flesh or around seed (jelly seed), or with spongy stem end (grey brown flesh/cavity at stem end).*
MINOR DEFECTS	
PHYSICAL / PEST DAMAGE	*With >5 attached scale insects.*
	With sooty mould (brown-black spotting) or bacterial spot (black spotting), not open or weeping, affecting in total >1 sq
	With pink spot >15 spots (each 5mm diameter) (former scale attachment areas).
	With shallow (<2mm), healed scarring, eg. hail or with cleavage scar affecting in aggregate >2 sq cm or with bruising affecting > 2 sq cm.; no deep scars or soft/moist deep-seated bruises.
TEMPERATURE INJURY	*With bleaching (sunburn) affecting >20% of visual surface; no grey-brown blotches or black depressed areas (sunscald).*
PHYSIOLOGICAL DISORDERS	*With pronounced/dark lenticels (>1-2mm wide) affecting >20% of skin; not star shaped or cracked.*
SKIN MARKS / BLEMISHES	*With sap burn or sap stain/spotting affecting >1sq cm.*
	With light blemish, eg. dense, thick russet lines affecting >6 sq cm; scattered thin lines are allowable.
	With dark blemish >1 sq cm (rub, abrasion damage).
CONSIGNMENT CRITERIA	
TOLERANCE PER CONSIGNMENT	*Total minor defects (within allowance limit) to be < 2 defects per item Total minor defects (outside allowance limit) must not exceed 10% of consignment. Total major defects must not exceed 2 % of consignment. Combined Total not to exceed 10%.*
PACKAGING & LABELLING	*Packaging manufactured from new food grade materials or sanitised returnable crates. All labelling must meet the current legislative requirements. Labelling to identify grower's name/brand (plus growers name/code if via a packhouse), address, contents, class, size and minimum net weight. Produce to identify 'Packed On' date (eg. Pkd*
SHELF LIFE	*Produce must provide not less than 14 days clear shelf life from date of receival.*
RECEIVAL CONDITIONS	*Compliance with Quarantine Treatments (if required) for Interstate Consignment. Stacked onto a stabilised pallet. Refrigerated van with air bag suspension, unless otherwise approved. Pulp Temperature 13 - 18 °C for Receival.*
CHEMICAL & CONTAMINANT RESIDUES	*All chemicals used pre/postharvest must be registered and approved for use in accordance with the requirements of the APVMA regulatory system. Residues, Contaminants and Heavy Metals to comply to the FSANZ Food Standards Code ML's and MRL's.*
FOOD SAFETY REQUIREMENTS	*Produce is to be grown and packed under a HACCP based food safety program that is subject to an annual third-party audit. A copy of current certification to be forwarded to receiver. Produce that meets the above specifications but is not grown under a HACCP based food safety program must not be labelled Class 1.*
Specifications reviewable: eg. to account for specific regional effects or adverse seasonal impacts on quality or early or late seasonal variances as agreed and communicated formally in writing.	

GENERAL

Mangos are grown in tropical and subtropical lowlands throughout the world. Mangos are one of the most consumed fruits worldwide and one of the most important fruit crops in tropical and subtropical areas of the world. Major production areas include India, Mexico, Brazil, and the Philippines. Other areas are Guatemala, Ecuador, Nicaragua, Peru, Haiti, Australia, South Africa, Israel, and Egypt.

The United States (U.S.) receives the majority of mangos from Mexico. Mexico produces many varieties but mainly exports five main varieties. The main varieties are Tommy Atkins, Haden, Ataulfo, Kent and Keitt. Mexican mangos are available from February through mid September.

Mangos cannot be shipped freely into the U.S. because of the possibility that insects and diseases could be introduced into the U.S., which could devastate domestic crops. Hot water treatment is one method used to treat mangos and safeguard against fruit flies from entering the United States.

Major subtropical pests of mangos are several species of fruit flies that are not native to the United States. Hot water treatment is a method used to treat mangos and safeguard against the entrance of fruit flies on U.S. soil.

REPRESENTATIVE SAMPLING

The importance of obtaining representative samples cannot be over emphasized. Accurate certification is possible only if the samples examined are truly representative of the entire lot or accessible portion. All portions of a lot or load should receive the same attention in sampling regardless of the difficulty involved in reaching all layers or parts of a lot or load. Anytime the entire lot requested is not accessible for sampling, the inspection and certificate must be restricted to the accessible portion.

Size of Sample

The tolerances in the U.S. Standards for Grades of Mangos are determined on the basis of count.

For packages with less than 25 fruit, the entire contents of the package shall be the sample. For packages with 25 or more fruit, the minimum sample size is 25 fruit. Whenever defects exceed the package in one or more samples in package with 25 or more fruit, the entire contents of at least one of those packages must be examined.

Sampling for Internal Defects

During examination for external defects it may be difficult for inspectors to detect the presence of internal defects. Some fruit must be cut from every sample. The number of specimens cut is discretionary and based on such factors as varietal characteristics, growing conditions, time of year, the ripeness of fruit and any external characteristics of *possible* internal defects.

When there are external indications of possible internal defects, then that fruit should be cut and the percentage of internal defects is based on the entire sample examined. It is not based upon the number of fruit cut. For example, if an inspector examines a 25-count sample of mangos, and after cutting 10 suspicious specimens finds 1 with an internal defect, the percentage of internal defects is 4% (1 defective fruit out of the entire sample, 25 fruit in this case).

Number of Samples

As a general rule a minimum of 1-1/2 to 2% of the lot must be examined. It is the inspector's responsibility to examine additional representative samples when the quality, condition, or size in samples is decidedly different to ensure an accurate description of the lot.

TOLERANCES AND APPLICATION OF TOLERANCES

The tolerances in the U.S. Standards for Grades of Mangos are based on count. Therefore, the determination of percentages must be made on that basis.

51.1044 *U.S. No. 1.* (a) *Tolerances.* In order to allow for variations incident to proper grading and handling, not more than 10 percent, by count, of the mangos in any lot may fail to meet the requirements of this grade, including in this amount not more than 5 percent shall be allowed for defects causing serious damage, including in this latter amount not more than 2 percent shall be allowed for decay.

§51.1045 *U.S. No. 2.* (a) *Tolerances.* In order to allow for variations incident to proper grading and handling, not more than 10 percent, by count, of the mangos in any lot may fail to meet the requirements of this grade, included in this amount not more than 2 percent shall be allowed for decay.

Summary of Tolerances

U.S. Fancy and U.S. No. 1.

Total Defects	10%
Including serious damage	5%
Including decay	2%

U.S. No. 2

Total Defects	10%
Including decay	2%

Application of Tolerances

§51.1046 *Application of Tolerances.* The contents of individual packages in the lot, based on the sample inspection, are subject to the following limitations: (a) For a tolerance of 10 percent or more, individual packages shall have not more than 1-1/2 times the tolerance specified, and for a tolerance of less than 10 percent, individual packages shall have not more than double the tolerance specified; *Provided*, That at least one defective specimen may be allowed in any package: *And provided further,* that the averages for the entire lot are within the tolerances specified for the grade.

U.S. Fancy and U.S. No. 1.

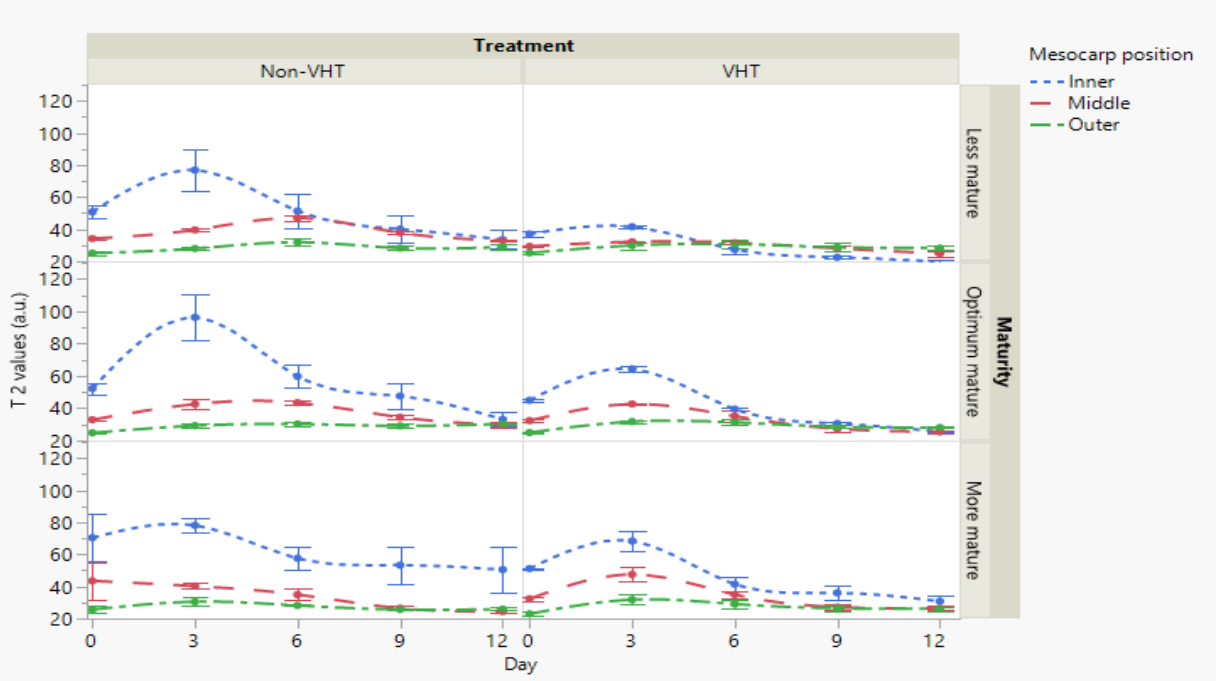
Total Defects	15%
Including serious damage	10%
Including decay	4%

U.S. No. 2

Total Defects	15%
Including decay	4%

Provided, That at least one defective specimen may be allowed in any package. *And provided further,* that the averages for the entire lot are within the tolerances specified by the grade.

Appendix 48: T2 values of inner, middle, and outer parts of ‘B74’ fruit mesocarp after VHT until the end of shelf-life for different fruit maturities: less: <15% DMC, optimum: 15 - 17% DMC and more mature: >17% DMC. Each data point is the average of three fruit for each fruit value calculated from two region of interest (ROI). Error bars show standard error of the mean. Graphic constructed using JMP Pro 16.0.0 (512257).



Appendix 49: PD values of inner, middle, and outer ‘B74’ fruit mesocarp at different stages of ripening with and without VHT for differentially mature fruit (less: <15% DMC, optimum: 15 - 17% DMC and more mature: >17% DMC). Each point is the average of three fruit. For each fruit, values were calculated from two region of interest (ROI). Error bars show standard error of the mean. Graphic constructed using JMP Pro 16.0.0 (512257).

