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**“Green Technologies in the Supply Chain of Agri-Food Company of kiwifruit
for Improved Resource Management and Fruit Quality”**

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Abstract

The agricultural sector plays a fundamental role in economic, social, and environmental impact, providing employment for over 44 million people in the EU. However, it faces growing challenges, including environmental degradation, overexploitation of resources and increasing demand for sustainable practices. In response, the EU Commission has adopted a package of measures to promote the sustainable use of key natural resources and strengthen the resilience of the EU's food systems and agriculture. These measures are expected to deliver long-term economic, social, health, and environmental benefits for all.

Against this background, this PhD thesis proposal, carried out in collaboration with Tre Colli S.r.l., a renowned kiwifruits producer located in Cisterna di Campoleone (Velletri, Rome, Italy), aims to address these pressing challenges by exploring innovative approaches to enhance digitalization and sustainability in the agri-food supply chain. The aim is to minimise environmental impact while improving efficiency and fruit quality. This study focuses on two different kiwifruit cultivars—*Actinidia chinensis* 'Soreli' (yellow fruit) and a newly developed red kiwifruit cultivar. By integrating advanced technologies and novel treatments, the research aims to optimize resource utilisation and improve fruit quality.

In particular, for the yellow cultivar 'Soreli', efforts are being made to mitigate critical environmental issues such as reducing pollution and minimising water waste —issues of growing importance in the modern agri-food landscape.

This study focused on integrating digital technologies and sustainable agricultural practices to optimize kiwifruit cultivation. The research examined the following key aspects:

1. **Digital Monitoring** – The deployment of remote sensing devices (*Tree Talker* sensors) in company orchards to assess plant health throughout the vegetative period.
2. **Water Deficit Treatments** – The application of varying irrigation levels (60%, 80%, and 100% of standard water volumes) in different orchard sections during the vegetative phase to evaluate whether controlled water stress could positively influence post-harvest fruit metabolism and quality.
3. **Post-Harvest Quality Assessments** – Evaluating the fruit from treated and control plants during storage to determine the impact of different irrigation strategies.

This study revealed valuable insights into the impact of optimized irrigation

strategies and technological innovations on kiwifruit cultivation. By integrating digital monitoring and sustainable water management practices, we demonstrated how resource efficiency can enhance plant health, improve fruit quality, and contribute to both economic and environmental sustainability. The main findings are summarized as follows:

- **Optimized Irrigation & Plant Health:** excessive irrigation did not always enhance plant performance. Vegetation spectral indices and fruit quality analyses indicated that deficit irrigation, particularly at 80% water availability, resulted in higher CVI (Chlorophyll Vegetation Index) and LNBI (Leaf Nitrogen Balance Index) values for leaves compared to full irrigation. This suggests that reducing water use can enhance vegetative health without compromising fruit quality.
- **Soil Sensors & Vegetation Correlation:** soil moisture data obtained from sensors correlated with vegetation indices, demonstrating the impact of water availability on plant health, growth, and productivity.
- **Sap Flow Monitoring:** Transpiration rates remained similar between the 100% and 80% irrigation treatments, with only a slight decline observed at 60% irrigation.
- **Post-Harvest Fruit Quality:** controlled reduced irrigation in ‘Soreli’ kiwifruit significantly improved fruit quality. Key attributes such as soluble solids content, firmness, yellow coloration, polyphenol and flavonoid levels, and antioxidant enzyme activity increased without negatively affecting weight loss (WL), titratable acidity (TA), or other bioactive compounds during storage.
- **Economic & Environmental Impact:** reducing irrigation by 20% (80% treatment) resulted in both economic savings and reduced water waste while maintaining high-quality produce. This demonstrates a viable approach for sustainable kiwifruit production, aligning with future climate adaptation strategies.

For the newly developed red kiwifruit cultivar, the company aimed to assess post-harvest preservation strategies and enhance red pigmentation.

The study explored the application of *cis-3-hexenyl butyrate* (HB), a ripening inducer, to evaluate its effects on fruit ripening, color intensity, sugar content, and anthocyanin concentration. Advanced, non-destructive techniques such as AOTF-NIR (Acousto-Optic Tunable Filter - Near InfraRed) have been used to confirm these results.

The main findings are summarized as follows:

- The study on red kiwifruits treated with HB reveals that treatment significantly influences the ripening process by accelerating sugar content increase, enhancing anthocyanin accumulation, and improving red coloration.
- NIR technology successfully discriminated anthocyanins and soluble solids content (SSC), with promising prediction models, especially when combined with Standard Normal Variate (SNV) filtering and Partial Least Squares (PLS) regression.

Overall, this research allowed the company to realize the application of sustainable processes, devices, and approaches within the industrial landscape, enhancing competitiveness and integrating the themes of environmental sustainability and digitalization in the agri-food.

The research on the new red kiwifruit cultivar focused on improving post-harvest preservation and enhancing fruit pigmentation. By applying innovative treatments and advanced analytical techniques, we gained valuable insights into the ripening process and fruit quality assessment. The key findings include:

- **Effect of HB Treatment:** HB significantly accelerated the ripening process by increasing sugar content, enhancing anthocyanin accumulation, and improving red coloration.
- **NIR Technology Advancements:** The AOTF-NIR system successfully differentiated anthocyanin content and SSC, with promising predictive models—especially when combined with SNV filtering and PLS regression.

Key words: Sustainability, Green Technologies, Digitalization, Kiwifruit Supply Chain, Kiwifruits

Riassunto

Il settore agricolo svolge un ruolo fondamentale nell'impatto economico, sociale e ambientale, fornendo occupazione a oltre 44 milioni di persone nell'UE. Tuttavia, si trova ad affrontare sfide crescenti, tra cui il degrado ambientale, lo sfruttamento eccessivo delle risorse e la crescente domanda di pratiche sostenibili. In risposta, la Commissione europea ha adottato un pacchetto di misure per promuovere l'uso sostenibile delle principali risorse naturali e rafforzare la resilienza dei sistemi alimentari e dell'agricoltura dell'UE. Si prevede che queste misure forniranno benefici economici, sociali, sanitari e ambientali a lungo termine per tutti.

In questo contesto, questa proposta di tesi di dottorato, realizzata in collaborazione con Tre Colli S.r.l., rinomato produttore di kiwi con sede a Cisterna di Campoleone (Velletri, Roma, Italia), mira ad affrontare queste sfide urgenti esplorando approcci innovativi per migliorare la digitalizzazione e la sostenibilità nella filiera agroalimentare. L'obiettivo è quello di ridurre al minimo l'impatto ambientale, migliorando al contempo l'efficienza e la qualità dei frutti. Questo studio si concentra su due diverse cultivar di kiwi: *Actinidia chinensis* 'Soreli' (frutto a polpa gialla) e una cultivar di kiwi rosso di recente sviluppo. Integrando tecnologie avanzate e nuovi trattamenti, la ricerca mira a ottimizzare l'utilizzo delle risorse e a migliorare la qualità dei frutti.

In particolare, per la cultivar gialla 'Soreli', si stanno compiendo sforzi per mitigare le criticità ambientali come la riduzione dell'inquinamento e la minimizzazione dello spreco di acqua, questioni di crescente importanza nel moderno panorama agroalimentare.

Questo studio si è concentrato sull'integrazione delle tecnologie digitali e delle pratiche agricole sostenibili per ottimizzare la coltivazione del kiwi. La ricerca ha esaminato i seguenti aspetti chiave:

1. **Monitoraggio Digitale** – L'implementazione di dispositivi di telerilevamento (sensori *Tree Talker*) nei frutteti aziendali per valutare lo stato di salute delle piante durante tutto il periodo vegetativo.
2. **Trattamenti del Deficit Idrico** – L'applicazione di livelli di irrigazione variabili (60%, 80% e 100% dei volumi d'acqua standard) in diverse sezioni del frutteto durante la fase vegetativa per valutare se lo stress idrico controllato possa influenzare positivamente il metabolismo e la qualità dei frutti post-raccolta.

3. **Valutazione della Qualità Post-Raccolta** – Valutazione dei frutti provenienti da piante trattate e controllate durante lo stoccaggio per determinare l'impatto delle diverse strategie di irrigazione.

Questo studio ha rivelato preziose informazioni sull'impatto delle strategie di irrigazione ottimizzate e delle innovazioni tecnologiche sulla coltivazione del kiwi. Integrando il monitoraggio digitale e le pratiche di gestione sostenibile dell'acqua, abbiamo dimostrato come l'efficienza delle risorse possa migliorare la salute delle piante, migliorare la qualità dei frutti e contribuire alla sostenibilità economica e ambientale. I principali risultati sono riassunti come segue:

- **Irrigazione Ottimizzata & Salute delle Piante:** un'irrigazione eccessiva non sempre ha migliorato le prestazioni delle piante. Gli indici spettrali della vegetazione e le analisi della qualità dei frutti hanno indicato che l'irrigazione deficitaria, in particolare con una disponibilità idrica dell'80%, ha portato a valori CVI (Chlorophyll Vegetation Index) e LNBI (Leaf Nitrogen Balance Index) più elevati per le foglie rispetto all'irrigazione completa. Ciò suggerisce che la riduzione dell'uso di acqua può migliorare la salute delle piante senza compromettere la qualità dei frutti.
- **Sensori del Suolo & Correlazione Vegetativa:** i dati sull'umidità del suolo ottenuti dai sensori sono correlati con gli indici di vegetazione, dimostrando l'impatto della disponibilità di acqua sulla salute, la crescita e la produttività delle piante.
- **Monitoraggio del Flusso di Linfa:** i tassi di traspirazione sono rimasti simili tra il 100% e l'80% dei trattamenti di irrigazione, con solo un leggero calo osservato al 60% di irrigazione.
- **Qualità della Frutta Post-Raccolta:** l'irrigazione controllata ridotta nel kiwi 'Soreli' ha migliorato significativamente la qualità della frutta. Attributi chiave come il contenuto di solidi solubili, la compattezza, la colorazione gialla, i livelli di polifenoli e flavonoidi e l'attività degli enzimi antiossidanti sono aumentati senza influire negativamente sulla perdita di peso (WL), sull'acidità titolabile (TA) o su altri composti bioattivi durante la conservazione.
- **Impatto Economico & Ambientale:** la riduzione dell'irrigazione del 20% (trattamento all'80%) ha comportato sia un risparmio economico che una riduzione degli sprechi idrici, pur mantenendo prodotti di alta qualità. Ciò dimostra un approccio praticabile per la produzione sostenibile di kiwi, in linea con le future strategie di adattamento ai cambiamenti climatici.

Per la cultivar di kiwi rosso di recente sviluppo, l'azienda mirava a valutare le strategie di conservazione post-raccolta e a migliorare la pigmentazione rossa. Lo studio ha esplorato l'applicazione del *cis-3-esenil butirrato* (HB), un induttore della maturazione, per valutarne gli effetti sulla maturazione dei frutti, l'intensità del colore, il contenuto di zucchero e la concentrazione di antociani. Per confermare questi risultati sono state utilizzate tecniche avanzate e non distruttive come AOTF-NIR (Acousto-Optic Tunable Filter – Near InfraRed).

I principali risultati sono riassunti come segue:

- Lo studio sui kiwi rossi trattati con HB rivela che il trattamento influenza significativamente il processo di maturazione accelerando l'aumento del contenuto di zucchero, migliorando l'accumulo di antociani e migliorando la colorazione rossa.
- La tecnologia NIR ha discriminato con successo antociani e contenuto di solidi solubili (SSC), con modelli di previsione promettenti, soprattutto se combinata con il filtraggio Standard Normal Variate (SNV) e la regressione Partial Least Squares (PLS).

Nel complesso, questa ricerca ha permesso all'azienda di realizzare l'applicazione di processi, dispositivi e approcci sostenibili all'interno del panorama industriale, aumentando la competitività e integrando i temi della sostenibilità ambientale e della digitalizzazione nel settore agroalimentare.

La ricerca sulla nuova cultivar di kiwi rosso si è concentrata sul miglioramento della conservazione post-raccolta e sul miglioramento della pigmentazione dei frutti. Grazie all'applicazione di trattamenti innovativi e tecniche analitiche avanzate, abbiamo acquisito preziose informazioni sul processo di maturazione e sulla valutazione della qualità dei frutti. I risultati principali includono:

- **Effetto del trattamento di HB:** HB ha accelerato significativamente il processo di maturazione aumentando il contenuto di zucchero, migliorando l'accumulo di antociani e migliorando la colorazione rossa.
- **Progressi della tecnologia NIR:** il sistema AOTF-NIR ha differenziato con successo il contenuto di antociani e il SSC, con promettenti modelli predittivi, soprattutto se combinato con il filtraggio SNV e la regressione PLS.

Parole chiave: Sostenibilità, Tecnologie Green, Digitalizzazione, Filiera del kiwi, Kiwi

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CHAPTER I

1. State of the Art

1.1. Italian agri-food supply chain and its impact on European Union economy

The industrial agriculture sector is recognized as a fundamental pillar of Europe's economy. Italy contributes 14% of the European Union's total agricultural production, playing a key role in several sectors. Notably, Italy holds a 37% share in wine industry, second only to France (43%), and a 33% share in olive oil production, following Spain's 48%. In fruit sector, Italy accounts for 18% of EU production, but faces strong competition from Spain, which leads with 28%. Additionally, Italy continues to excel in secondary agricultural activities and services, which together constitute 18% of the country's total agricultural output.

From an agri-food industry perspective, Italy ranks third among EU countries, demonstrating a stronger growth trend compared to its main competitors. Contributing approximately 12% of the total value added—positioned behind Germany and France but ahead of Spain—Italy stands out as the undisputed leader in the pasta industry, generating over 73% of the EU's total revenue. Additionally, Italy holds a significant market share in the wine sector (28%), bakery products and biscuits (21%), as well as in processed fruit and vegetables, the coffee, tea, and herbal infusion industry, and the milling and rice sector, each accounting for 17% of the European market turnover.

In 2022, Italy's food supply chain, encompassing both agricultural production and food processing, generated a total value added of €64 billion, with €37.4 billion from agriculture and €26.7 billion from the food industry. The sector contributed 3.7% to Italy's total economic value added, which increased to 7.7% when downstream activities, such as food distribution and catering, were considered. Moreover, when factoring in essential services and operations that bring products from farm to table—such as transportation, logistics, and trade intermediation—the agri-food sector's estimated contribution to GDP exceeded 15.2% (Palmieri et al., 2023).

1.2. Industrial agriculture practices and their effects on environmental sustainability

Agriculture encompasses the cultivation of food and related products through farming practices, and it plays a pivotal role in ensuring survival and fostering the well-being of societies. From its origins in the simple act of gathering food, agriculture has evolved into the complex and sophisticated industry we know today. However, in recent decades, the industrialization of agriculture has triggered significant environmental and ecological challenges have impacted both developed and developing nations (Nagendran, 2011).

This progression toward industrialized agricultural systems is defined by market-oriented production, extensive use of chemical inputs, high energy consumption, and significant water use, with the overarching goal of maximizing productivity. However, in recent years, these practices have contributed to a range of negative impacts, including environmental pollution, climate change, and the loss of biological and ecological diversity (Bonciarelli et al., 2016; Veerachamy et al., 2022).

1.2.1. Biological and ecological diversity

The concept of diversity in agriculture encompasses all plants, animals, and microorganisms that coexist and interact within croplands and their surrounding environments. Ecological diversity forms the backbone of agricultural systems, directly influencing productivity, resilience, and sustainability. Biodiversity is a critical component in agriculture, underpinning the resilience of food systems in the face of environmental disturbances (Simmons, 1999). A diverse agricultural ecosystem enhances soil health, reduces reliance on chemical inputs, and helps to mitigate the effects of extreme weather events. Biodiversity also supports essential ecosystem services, such as pollination, pest control, and nutrient cycling, while providing genetic resources crucial for adapting to environmental changes and evolving societal needs (Altieri & Rogé, 2009).

Emphasizing biodiversity in agricultural practices not only strengthens environmental sustainability but also aligns with global efforts to address resource scarcity and climate change (Zimmerer, 2010).

1.2.2. Climate change

Climate is the most critical factor influencing agricultural production, making the sector highly sensitive to climatic variations. However, climate change, largely driven by unsustainable agricultural practices, will intensify these challenges by reducing access to drinking water, harming human health, and posing a significant threat to food security in numerous countries. The vulnerability of agricultural productivity to climate change is well-documented, with research highlighting that, without effective response strategies, the sector will face increasingly severe consequences. Key areas of study include the physical effects of climate change on agriculture, such as shifts in crop and livestock yields, and the economic repercussions of these changes (Cresser et al., 2008).

Projected impacts of climate change include more frequent and intense droughts, flooding, and storm damage. Climate change is also expected to lead to long-term shortages of water and other essential resources, degradation of soil quality, intensification of drought and desertification, as well as outbreaks of diseases and pests affecting both crops and livestock, and rising sea levels. These challenges are likely to result in significant declines in agricultural productivity, particularly in vulnerable regions, primarily due to reduced crop yields. To address these urgent issues, there is an increasing focus on improving agricultural practices through sustainable approaches aimed to mitigate the impacts of climate change. The World Bank is actively supporting initiatives to promote climate-smart agriculture, advance sustainable farming techniques, and implement policies designed to build resilience while minimizing the environmental footprint of the agricultural sector (Kurukulasuriya & Rosenthal, 2013).

1.2.3. The impact of sustainable farming techniques on biodiversity and climate mitigation

The literature highlights that the social factors contributing to environmental degradation are becoming more apparent, representing a pressing issue that affects nearly every nation. The primary sources of pollution in the agricultural sector include waste generated from farming activities (Nagendran, 2011), excessive water usage (Ghimire et al., 2021) and high energy consumption (Inumula et al., 2020). These factors place significant pressure on natural resources such as water and land, which are extensively used in agricultural practices (Preite et al., 2023).

To address the negative impacts of highly productive but intensive farming, among the various goals that will need to be achieved in the 21st century, there are food security, improved nutrition, and the promotion of sustainable agriculture in a position to contribute to the long-term well-being of the population by producing sufficient food, goods, and services in an economically efficient and profitable manner, while being socially responsible and environmentally respectful (Chasek et al., 2016; Czekala & Pulka, 2024). Today's consumers are increasingly focused on the quality of agri-food products, linking it to the environmental and social sustainability of production. This results in growing demand for the healthiness and genuineness of food products, alongside a reduction in climate pollution and energy overconsumption (Briamonte, 2024).

In 2018, the Food and Agriculture Organization (FAO) outlined five key principles of sustainable agriculture, which remain fundamental guidelines to this day. These principles are as follows:

- Enhancing productivity, employment, and value addition in food systems; this involves transforming agricultural practices to ensure stable food supply chains while minimizing water and energy consumption.
- Protecting and optimizing natural resources, focusing on environmental conservation, reducing water pollution, preventing habitat and ecosystem destruction, and mitigating soil degradation.
- Improving livelihoods and fostering inclusive economic growth to ensuring fair income for farmers that covers production costs and guarantees a decent standard of living.
- Strengthening adaptation of production models to mitigate the adverse effects of weather events caused by climate change and market price fluctuations.
- Adapting governance to emerging challenges through regulatory frameworks that ensure transparency and equity while enabling a balance between the public and private sectors (Fattorini, 2023).

The term “Sustainable Agriculture” refers to the ability of agriculture to contribute in the long term to welfare of people by producing sufficient food, goods, and services in an economically efficient and profitable manner, while being socially responsible and environmentally respectful. Agriculture places great emphasis on cross-cutting issues such as food security, production traceability, product quality, and respect for the environment and human resources (Briamonte, 2024).

Indeed, the environmental and sustainability challenges are driving the need for new systems of agricultural labour management that not only improve efficiency but also reduce the impact of this crucial activity (Arrieta et al., 2020).

1.2.4. Water management as a sustainable strategy to support agriculture

The improvement of profitability in the agricultural industry is crucial for ensuring the economic sustainability of farms, enhancing market competitiveness, and supporting the vitality of rural communities. Greater profitability allows agricultural businesses to invest in innovation, adopt sustainable practices, and address challenges related to climate change and market fluctuations (Fattorini, 2023).

Profitability in agriculture can be enhanced through three main strategies: increasing agricultural yields, reducing production costs and improving crop quality, all while minimizing environmental impact. These strategies can be implemented by optimizing water and energy consumption (Briamonte, 2024).

Competition for freshwater resources has intensified over the decades due to population growth, economic expansion, and the demand for agricultural products, both for food and non-food uses. The challenges related to freshwater scarcity and pollution are expected to worsen in the future due to a significant increase in water demand coupled with a decrease in both availability and quality. It is estimated that our dependency on water resources will increase significantly in the future and this brings problems for food security and environmental sustainability (Ercin & Hoekstra, 2014).

Irrigation in agricultural ecosystems accounts for most of the world's freshwater consumption (Arrieta et al., 2020), with these ecosystems using about 70% of available water, most of which is directed toward irrigation (Boretti & Rosa, 2019). Globally, irrigated agricultural land amounts to 275 million hectares, and it increases by 1.3% each year. Although irrigated land represents only 23% of cultivated land, it contributes to 45% of total food production. Projections suggest that to meet the food demand of 2050 global water usage will need to increase by 53%, with developing nations requiring a 50% increase and industrialized countries 16%, assuming current productivity and technological advancements remain unchanged. Therefore, while irrigation is essential for sustaining plant growth and productivity, optimizing its use is essential for ensuring long-term sustainability (Velasco-Muñoz et al., 2019).

To achieve this objective, it is necessary to analyze the whole irrigation process, starting with the water source and extending to its application in agriculture. Irrigation can be divided into three key stages. The first stage involves drawing water from its source and conveying it through channels to the designated area. The second stage focuses on delivering water to the root zone, facilitating crop absorption—this includes both conventional methods, such as flood and furrow irrigation, as well as advanced techniques like drip systems and microsprinklers. The final stage encompasses the entire plant growth process, where water moves from the roots to other parts of the plant. The primary objective is to optimize water use efficiency and minimize losses throughout all three stages (Zhang et al., 2019). In fact, excessive irrigation can lead not only to the waste of this vital resource but also to detrimental effects both for the plant itself and the products it generates; this as has been demonstrated for various horticultural crops, such as kale (Brazel et al., 2023), kiwifruits (Lembo et al., 2023) and pear (Kalcsits et al., 2023).

1.2.4.1. Deficit Irrigation

For the reasons mentioned above, it is essential to focus on minimizing water consumption, the most valuable resource on Earth, necessary for the survival of living organisms and the functioning of the biosphere (Czekala & Pulka, 2024).

The use of water in crops involves two main factors: one is the water lost through evaporation from both the soil and the plants, known as evapotranspiration (ET), and the other includes the losses from water distribution to the field. Irrigation water contains salts, and as it evaporates, these salts accumulate in the soil and must be moved below the root zone to prevent concentrations that could hinder crop growth. This movement of salts is facilitated by applying water in excess of ET, a process called salt leaching. While some water loss is inevitable to maintain the balance of salts, it can be minimized by using efficient irrigation techniques and proper management. When water supply is limited and transpiration decreases below what is needed based on environmental evaporation rates, it leads to reduced biomass production.

Traditionally, the standard irrigation approach was to ensure that crops received enough water to meet their maximum transpiration potential, satisfying full ET needs throughout the growing season. However, this method is increasingly being questioned in areas with water scarcity due to the large volumes of water required and the negative environmental impact.

As a result, a shift in irrigation strategy is occurring, providing water below the required ET level, which in turn lowers overall water demand for irrigation; this practice is referred to as deficit irrigation (DI) (Feres & Soriano, 2007; Payero et al., 2009).

Thus, DI is a practice in which less water is supplied to the plant than that of the expected evapotranspiration, an important parameter for assessing crop water requirements and managing water resources in agriculture. The inherent goal of deficit irrigation is to enhance production efficiencies and improve fruit quality (Reid & Kalcsits, 2020), and save irrigation water. This has been demonstrated in the study by Cui et al. (2008), where DI saved 50% of irrigation water, increased the ratio between yield and total irrigation water greatly without yield reduction and improved berry quality and taste of grapevine.

Three main DI strategies can be mentioned:

- *Regulated Deficit Irrigation* (RDI) is a water management strategy that involves the controlled application of reduced irrigation volumes during specific phenological stages of crops to optimize yield and product quality while minimizing water consumption. This approach is based on an understanding of plant water requirements, restricting irrigation during less sensitive growth phases, and ensuring adequate supply during critical periods for fruit development. RDI is implemented in fruit orchards and vineyards to enhance crop quality by increasing sugar concentration, aroma, and pigment accumulation, while also promoting better root development and improving water use efficiency (Yang et al., 2022).
- *Sustained Deficit Irrigation* (SDI) is a water management strategy that involves continuously applying irrigation at levels below full crop water requirements throughout the entire growing season. Unlike RDI, which adjusts water supply based on specific phenological stages, SDI maintains a consistent water deficit, encouraging plants to adapt to limited water availability. This technique aims to improve water use efficiency, control excessive vegetative growth, and enhance fruit quality parameters such as sugar concentration, firmness, and secondary metabolite accumulation. However, it is necessary to monitor the process to prevent severe stress that could negatively impact yield and overall plant health. SDI is commonly used in perennial crops like vineyards and orchards in arid and semi-arid regions to optimize water resources and to maintain acceptable productivity levels (Galindo et al., 2018)

- *Partial Rootzone Drying* (PRD) is an advanced irrigation technique that involves alternately watering only one side of the root zone while allowing the other side to dry out before switching irrigation to the opposite side. This method induces a physiological response in plants by stimulating the production of abscisic acid, which helps regulate stomatal closure, reducing transpiration without significantly affecting photosynthesis. PRD enhances water use efficiency, improves drought tolerance, and maintains or even enhances fruit quality by increasing sugar accumulation, color development, and secondary metabolite synthesis. It is commonly applied in viticulture and fruit orchards and requires precise irrigation scheduling to avoid excessive stress while optimizing crop performance under limited water conditions (Iqbal et al., 2020; Sepaskhah & Ahmadi, 2010).

Several studies have already tested deficit irrigation on different fruit plants and different results have been obtained depending on when the irrigation was initiated (H. Caspari, 1993; H. W. Caspari et al., 1994; Hsiao, 1973) and the type of fruit subjected to this water stress (H. W. Caspari et al., 1994; Ebel et al., 1995; Mitchell et al., 1989).

Despite the different results obtained, numerous studies have shown that deficient irrigation did not negatively affect fruit quality at harvest or return bloom for the following season, as demonstrated in our studies on the ‘Soreli’ kiwifruit cultivar (Asgharinia et al., 2023; Caspari et al., 1994; Lembo et al., 2023; Reid & Kalcsits, 2020).

In addition to the interest focused on controlled deficit irrigation to assess the effect on plants health and fruits quality during their storage (Lagos et al., 2017; Lembo et al., 2023; Zheng et al., 2023; Albergaria et al., 2020; Manda et al., 2021), in order to optimize water use, researchers, especially in recent years, are focusing on the implementation of systems with the aim of managing irrigation systems using IoT technologies and monitoring endogenous and exogenous crop factors. Machine learning algorithms are used to optimize these systems.

Furthermore, with the aim of safeguarding water, innovative systems are studied to recover water (Preite et al., 2023).

1.2.4.2. Drip Irrigation

One of the most widespread systems is “Drip Irrigation”, an efficient method that delivers water directly to plant roots through a network of pipes, valves, and emitters, allowing for a slow and precise release of water. This system enables significant water savings, reducing consumption by 40% to 70% compared to traditional methods, while enhancing nutrient efficiency through the precise application of fertilizers directly to the root zone and minimizing the incidence of fungal diseases by limiting moisture on the foliage (Goldberg et al., 1976). It is suitable for various types of crops, including vegetables, orchards, and vineyards, and can be implemented in different soil types and climatic conditions. Additionally, it can be easily automated, optimizing irrigation management and reducing manual labor (Taylor & Zilberman, 2017).

A drip irrigation system consists of a water source, which may be a well, a reservoir, or a municipal supply, a filtration system to remove particles and debris, preventing emitter clogging, distribution pipes that transport water from the source to the emitters that release water slowly and precisely into the root zone, and control valves that regulate water flow, either manually or automatically. The adoption of drip irrigation represents a sustainable and effective solution for modern agriculture, contributing to water conservation and optimizing crop productivity (Reddy et al., 2023).

1.2.4.3. Micro-Sprinkler Irrigation

“Micro-Sprinkler Irrigation” is another irrigation system which is becoming a preferred method for applying water in orchards because provides precise control over water distribution to optimize tree water requirements, ensuring efficient water use and reducing environmental impact. The irrigation scheduling of micro-irrigation systems is predominantly based on a water budget approach, which aims to maintain optimal soil moisture levels within the root zone, thereby minimizing both water stress and deep percolation losses. However, in localized micro-irrigation setups, accurately assessing the various components of the water balance remains a challenge, necessitating detailed soil moisture monitoring to obtain precise estimations of actual tree water consumption.

This irrigation integrates the advantages of drip irrigation while covering a broader soil surface area, facilitating a more extensive root system development.

This enhances nutrient and water availability, particularly during peak water demand periods. The technique is especially beneficial for soils with low permeability or limited water retention capacity, as well as on sloped terrains prone to runoff.

Similar to drip irrigation, micro-sprinklers are engineered for high-frequency irrigation with controlled application rates to prevent surface ponding.

A notable advantage of micro-sprinklers lies in their ability to prevent localized salinity buildup, as salinity levels tend to remain low near the soil surface and gradually increase with depth. However, challenges associated with micro-sprinkler systems include potential water losses and variability in distribution uniformity. Despite these limitations, research on various micro-sprinkler designs is demonstrating effective and uniform soil moisture distribution within the root zone, supporting plant growth and improving overall irrigation efficiency.

1.2.4.4. Sprinkler Irrigation

Sprinkler irrigation, also known as mist or spray irrigation, is a technique that distributes water in fine droplets, resembling light rainfall, using sprinklers or misting nozzles (Figure 1.1.) (Asgharinia et al., 2023; Lembo et al., 2023).



Figure 1.1. Sprinkler irrigation system in action at Tre Colli S.r.l, located in Cisterna di Campoleone (Velletri, Rome, Italy)

This method helps maintain consistent humidity levels, lower air temperature and provide plants with the necessary water supply. It has also been shown that the sprinkler water is more uniformly distributed within the root zone than that measured on the surface (Dwomoh et al., 2023; Li, 1998; Li & Liu, 2020).

It can be defined as an irrigation system that distributes water onto the field by projecting water from the nozzle of a sprinkler into the air, subsequently falling as discrete droplets of different diameters. The practicality of its use on various soils, climates, and crop types and in difficult topographic conditions is an attribute that makes sprinkler irrigation attractive. Sprinkler systems are used for crop and soil cooling, frost protection, delaying fruit and bud from developing, providing water for germinating seeds, application of agricultural chemicals, and land application of wastewater. Water-saving sprinkler irrigation requires water to be distributed uniformly and efficiently with very minimal losses (Dwomoh et al., 2023). Water-efficient sprinkler irrigation systems are designed to distribute water evenly across the field, minimizing waste. The sprinkler head plays a critical role in ensuring efficient water use by dispersing water throughout the area.

There are two primary categories of sprinkler systems available on the market today: rotating and fixed-head sprinklers. Rotating sprinklers encompass several types, including impact, gear-driven, reaction, and fluidic models.

Fixed-head sprinklers, on the other hand, generally refer to the various spray-type sprinklers that are commonly in use (Jin & Z. Wu., 2022; H. Li et al., 2021).

1.2.4.5. Effects of overwatering and underwatering on plants and fruit

A drastic overwatering and underwatering for crops are negative factors, leading to damage not only to the plants but also to the final product. In fact, it seems that effect of flooding, due to either meteoric events or excessive or wrong watering management, has been examined in view of the high sensitivity of kiwifruit roots to low oxygen availability (Bardi, 2020). Therefore, an excess of water can negatively influence the cultivation and quality of this fruit (Li et al., 2024).

While water supply is essential for kiwi cultivation, the plant exhibits high sensitivity to root waterlogging and anoxic soil conditions, which can result in weakened growth and increased susceptibility to pathogenic attacks, ultimately leading to plant decline. In fact, kiwi is notably vulnerable to water stress, largely due to its limited capacity for stomatal regulation. (Medda et al., 2022).

So, drastic stress is not beneficial for plant health and fruit quality, but a controlled deficit can provide benefits.

Extensive research has demonstrated that reducing irrigation water for fruits such as apples, citrus, mangoes, plums, and tomatoes improves overall fruit quality by enhancing sugar content and extending shelf life (Bai et al., 2023; Hassan et al., 2021; Zhu et al., 2016).

Moreover, parameters such as abscisic acid levels, total phenols, and anthocyanins have been shown to increase in plums, while in cherry tomatoes, antioxidant activity, lycopene, β -carotene content, and the intensity of red color and tomato flavor have also been shown to improve (Hassan et al., 2021; Lipan et al., 2021).

Another study, focused on the kiwi plant, examined the reduction of soil moisture on the morphological and physiological indicators of the Xu Xiang soft date red kiwifruit cultivar, demonstrating that a drastic reduction in soil moisture led to changes in morphological indicators such as plant height and diameter, as well as physiological indicators such as stomatal conductance of CO₂, photosynthetic rate, and chlorophyll content, ultimately affected the plant's morphological structure (Wu & Zhu, 2022).

Regarding grapevine (*Vitis vinifera* L.), minor water supplementation can boost yields and help maintain or even enhance berry quality. However, excessive irrigation may encourage overly vegetative growth, reduce berry pigmentation, lower sugar content if applied later in the growing season, and negatively impact wine quality. Additionally, a larger canopy area can lead to increased transpiration losses and heightened susceptibility to diseases, particularly fungal issues (Costa et al., 2007).

Multiple results with the cultivars ‘Fuji’ and ‘Gala’ showed that controlled reduction of water allowed for water savings of about 45–50% and 25–75% respectively, without affecting yield or fruit size compared to the full irrigation. Reducing water availability accelerated apple ripening, enhanced flesh firmness, and increased total soluble solids and aroma volatiles, both during the ripening phase and after storage (Leib et al., 2006; Einhorn & Caspari, 2004).

Regarding fruits such as peach (*Prunus persica* L.), Asian pear (*Pyrus serotina* L.), and prunes (*Prunus domestica* L.), it was observed that low water use reduced vegetative growth without negatively affecting yield. In contrast, in nut crops like almonds (*Prunus dulcis* Mill.), water stress decreased kernel yields by about 10 to

20% (Romero et al., 2004).

Cui et al. (2008) evaluated the effect of the irrigation deficit on field-grown pear-jujube trees. Treatments included different levels of water restriction applied during key growth phases (bud burst to leaf expansion, flowering to fruit set, fruit enlargement, and fruit ripening). All deficit irrigation strategies led to a 5–18% reduction in water usage and a 13–25% decrease in irrigation demand compared to full irrigation. Moderate and severe water limitations during the bud burst to leafing and fruit ripening stages enhanced fruit yield by 13.2–31.9% and 9.7–17.5%, respectively. During the bud burst to leaf expansion stage, moderate and severe water deficits did not impact fruit quality but significantly conserved water while boosting yield. A slight water deficit during the fruit enlargement stage, as well as mild to severe deficits during ripening, had no noticeable influence on fruit weight or volume. However, they slightly lowered fruit water content, contributing to a marked decrease in fruit spoilage during post-harvest storage. Additionally, these water-saving treatments accelerated the ripening process by 10–15 days and increased the fruit's market value. Improvements in fruit firmness, soluble solids concentration, sugar-to-acid ratio, and vitamin C levels were also observed due to deficit irrigation.

These results suggest that, while excessive or extreme water stress can be detrimental to both plant health and fruit quality, a well-managed water deficit can serve as a strategic tool to optimize agricultural production. Controlled irrigation reductions enhance various fruit quality parameters, improve resource efficiency, and even extend shelf life. The effects of water limitation, however, are highly species-specific, requiring careful calibration based on crop type, growth stage, and environmental conditions. Future research should continue to refine irrigation strategies to balance sustainability, productivity, and fruit quality while mitigating potential risks associated with water scarcity and climate variability (Ruiz-Sanchez et al., 2010).

1.2.5. Advancements in Precision Farming Technologies

One of the challenges for sustainable agriculture lies in achieving optimized yield and increasing farm income with a minimum of resource and financial inputs, while preserving the environment and this can be achieved through the advent of precision agriculture, replacing traditional practices (Blasch et al., 2022).

This approach to farming management, which has been evolving since the 1990s,

focuses on identifying and addressing the variations within and between fields to maximize profitability, sustainability, and environmental protection.

The adoption of this strategy is made feasible by advancements in technologies such as geographic information systems, global navigation satellite systems, remote sensing, satellite imagery, ground sensors, as well as mobile computing and telecommunication. These technologies enable farmers to map and analyse field conditions and apply tailored farming practices like tilling, seeding, fertilization, pest control, irrigation, harvesting, and livestock care to enhance efficiency (Blasch et al., 2022; Paxton et al., 2011).

The spread of digital technologies is transforming the industrial approach through the development of intelligent processes, devices, materials, equipment, and tools, as well as production systems and supply chains, leading to significant changes in business models, increasingly based on relational product-service system logic.

In horticulture field, digitalization brings benefits such as increased effectiveness and efficiency in food traceability processes, sales growth, and the use of data as a valuable knowledge asset for businesses (Duncan et al., 2021)

With the advent of precision agriculture, conventional methods are undergoing a revolution, integrating advanced technologies and tools. This shift is characterized by the strategic application of technology to optimize agricultural processes (Veerachamy et al., 2022). These intelligent farming systems has the potential to enhance crop yields for profitable agriculture, streamline agricultural transactions, and drive advancements in smart automation by data acquisition, monitoring, computerizing and data analysis in real-time (Arrieta et al., 2020).

1.2.5.1. TreeTalker devices and their role in the Internet of Things for smart agriculture

Several researchers have implemented systems using Internet of Things (IoT) in field, offering a significant opportunity to enhance environmental monitoring at minimal costs. This approach involves embedding unique identifiers into objects, animals, or individuals, enabling them to transmit data through a network without the need for direct human interaction. The concept has emerged from the integration of wireless technologies, microelectromechanical systems, and the Internet. These innovations have paved the way for real-time data transmission and the establishment of numerous affordable monitoring points in environmental

observation (Valentini et al., 2019)

An example is the TreeTalker© devices (TT), which are capable of measuring sap flow as an indicator of tree transpiration and xylem transport functionality (Figure 1.2). They can also assess tree radial growth using an infrared-based distance sensor. Additionally, they measure xylem moisture content as a gauge of hydraulic functionality and monitor tree stability parameters to enable real-time predictions of potential tree falls.



Figure 1.2. Sap flow probe of Tree Talker (TT) system in action at Tre Colli S.r.l, located in Cisterna di Campoleone (Velletri, Rome, Italy)

The devices provide multi-spectral measurements of sunlight transmitted through the canopy, spanning 12 bands across the visible and near-infrared spectra (Figure 1.3.), as well as track microclimatic parameters and transmit data in near-real time. Furthermore, these tools can measure physical environmental variables such as temperature and air and soil humidity (Sala et al., 2019).



Figure 1.3. Kiwi plant leaves monitored by TT device equipped with spectrophotometer with 12 spectral bands Tre Colli S.r.l, located in Cisterna di Campoleone (Velletri, Rome, Italy)

These devices, mainly used in the forestry field (Cocozza et al., 2023; Sala et al., 2019; Paxton et al., 2011; Tomelleri et al., 2022), seem to have excellent applications also in the fruit and vegetable field, as demonstrated by the monitoring of ‘Soreli’ kiwi plants (Asgharinia et al., 2023; Lembo et al., 2023). This allows farmers to use a mobile application remote monitoring to control air humidity, air temperature, and soil moisture-factors (Figure 1.4.) that can affect plant growth and health. (Abdullah et al., 2021; Lembo et al., 2023; Loukatos et al., 2022).

It involves the use of data-driven tools like GPS (Yousefi & Razdari, 2015), sensors (Asgharinia et al., 2023; Kumar & Ilango, 2018; Lembo et al., 2023; Veerachamy et al., 2022) and advanced analytics to monitor and manage crop growth, soil conditions, and resource inputs with high accuracy (Asgharinia et al., 2022; Shah & Wu, 2019). This approach aims to improve efficiency, reduce waste, and increase productivity while minimizing environmental impact. Given the importance of this type of technology in the socioeconomic environment of the country, this project presents our first advances in research on the design and implementation of a smart plant monitoring system based on IoT (Asgharinia et al., 2023; Lembo et al., 2023).



Figure 1.4. Soil moisture sensor placed 30 cm underground at root system level of ‘Soreli’ kiwi plants located in Tre Colli S.r.l, located in Cisterna di Campoleone (Velletri, Rome, Italy)

1.2.6. Non destructive methods for fruit maturity assessment

Fruit quality is a multifaceted concept that encompasses not only sensory attributes like appearance, texture, and flavor but also factors such as composition, nutritional value, storability, and the absence of defects. Various stakeholders in the fruit production chain assess quality based on their specific needs. For growers, aspects like production yield, orchard management, and disease resistance are crucial, whereas distributors prioritize fruit freshness and shelf life. The fruit quality undergoes variations depending on variability of biochemical composition (sugars, pigments, phenolic and aroma compounds), physiological response (ethylene production, respiration rate) and the physiological effect with the change of these quality traits during fruit growth, ripening and post-harvest period. Moreover, the fruit quality is dependent on the environment in which plants are grown, cultural practices and post-harvest fruit management. Estimating pre-harvest fruit quality and maturity is crucial for growers to determine optimal harvest timing, storage needs, and crop profitability (Bureau, 2009).

For instance, Azadnia et al. (2023) observed that the irregular flowering period of hawthorn trees resulted in fruit ripening at different times, which can negatively impact the marketability of harvested hawthorn fruits, resulting in reduced economic value and increased product waste.

Regarding apples, the fourth most widely produced and consumed fruit globally, harvest timing is a critical factor in ensuring high fruit quality for consumers. Both early and late harvests can negatively impact fruit quality (Musacchi & Serra, 2018). For avocado fruit, popular due to increasing consumer awareness of its nutritional benefits, determining the optimal ripeness stage for harvesting is crucial. Key quality attributes such as appearance, shape, size, firmness, flavor, and nutrient composition are established at harvest. Therefore, accurately determining the maturity stage is crucial in ensuring postharvest quality that meets consumer expectations. In many avocado cultivars, harvesting begins once the fruit has reached a minimum maturity level, defined by its ability to ripen without shriveling. If harvested prematurely, avocados tend to shrivel during storage, ripen unevenly, and develop undesirable characteristics, resulting in product waste (Magwaza & Tesfay, 2015).

This much waste in agricultural products can be largely controlled, and the products and water can be saved through proper harvesting, transportation, warehousing and processing methods (Islam et al., 2024).

However, most of the available investigative methods to determine the fruit maturity index are based on the complex processing of samples, using expensive chemical reagents that involving a considerable amount of manual work, resulting slow and destructive (Moghimi et al., 2010).

For example, sugar concentration is considered a key indicator of fruit maturity but is generally evaluated using destructive tools such as refractometer (Pasquariello et al., 2013; Petriccione et al., 2015), hydrometer (Magwaza & Opara, 2015), Electronic Tongue (Németh et al., 2019) and High-Performance Liquid Chromatography (HPLC) (Obón et al., 2011). Returning to our previous example of avocado fruit, mesocarp oil content and dry matter gradually increase during fruit growth and these are significantly correlated with maturity and eating quality. Although these parameters are commercially valuable indicators, the methods used to measure them are destructive, time-consuming, costly, and require meticulous sample preparation (Magwaza & Tesfay, 2015).

The use of destructive methods like those previously mentioned to assess fruit maturity prior to harvest presents several drawbacks. These techniques require sampling that permanently damages the fruit, reducing potential yield and generating waste. Additionally, destructive testing is labor-intensive and time-consuming, limiting its feasibility for large-scale assessments. (Goffi et al., 2021; Islam et al., 2024; Jaywant et al., 2022).

Beyond being labor-intensive and destructive, these methods pose the risk of growers delivering inconsistent fruit quality to the market due to variability within a batch. Typically, destructive measurements rely on a limited number of representative samples to assess the maturity of an entire batch, which may not accurately reflect overall quality. Immature fruit within a batch can lead to uneven ripening, consumer dissatisfaction, and potential declines in future purchases (Magwaza & Tesfay, 2015).

A study by Donetti & Terry (2014) identified intra-varietal differences in fruit composition and challenges in predicting and controlling ripening patterns as key concerns among consumers and importers. To enhance competitiveness and maintain consumer confidence, the agri-food industry must ensure a consistent supply of high-quality fruit with uniform and predictable ripening.

For these many reasons developing objective, rapid, and non-destructive techniques for accurately determining individual fruit maturity is essential. Non-destructive analytical tools allow the simultaneous measurement of qualitative, quantitative, chemical and physical data from agro-food products, carrying out surface testing of fruits and vegetables without any intrusive technique affecting the food aspect and quality (El-Mesery et al., 2019).

These techniques enable the assessment of produce characteristics without causing damage, facilitating continuous monitoring throughout the production chain—from harvest to distribution. This approach is particularly beneficial in ensuring that products reach consumers while maintaining high-quality standards (Anjali et al., 2024).

In recent years, extensive efforts have been made to develop reliable non-destructive technologies for assessing fruit quality and maturity (Figure 1.5.). Prominent examples of such techniques include near-infrared spectroscopy (NIR) (Jie et al., 2014), nuclear magnetic resonance (NMR) (Si et al., 2022), hyperspectral imaging (Pu et al., 2015), thermal imaging, image scanning (Anjali et al., 2024),

electronic noses (da Silva Ferreira et al., 2023), Fourier transform NIR spectroscopy with attenuated total reflection (ATR-FTIR) (Bureau et al., 2009).

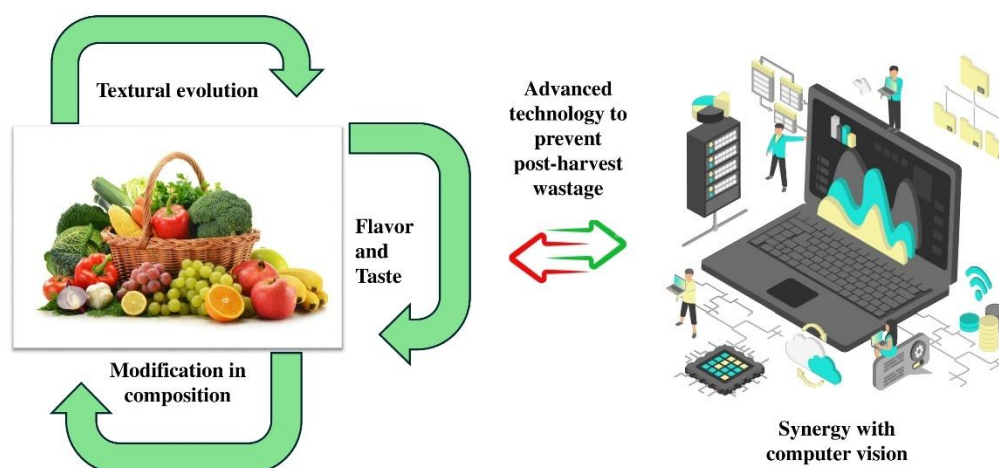


Figure 1.5. A representation of the evolutionary dynamics in fruits and vegetables, exploring textural evolution, changes in flavor and taste, and modifications in composition. The use of advanced technology to prevent post-harvest wastage is highlighted, synergistically connected to 'computer vision' for optimizing quality monitoring and management

1.2.6.1. Spectroscopic techniques as a non-destructive method

Spectroscopic techniques rely on the interaction between electromagnetic radiation and biological tissues. The electromagnetic spectrum covers a vast range, from rays with wavelengths around 10-12 meters through X-rays, ultraviolet, visible, infrared, microwaves, and finally, radio waves, which can reach wavelengths as long as 103 meters. In fruit quality assessment, the most commonly utilized wavelength ranges include visible light (400–800 nm), ultraviolet (4–400 nm), and near-infrared (750–2500 nm). When light strikes the fruit, various phenomena occur, including reflection, absorption, transmission, and scattering. Only a small fraction of the incident radiation is reflected at the surface, while the majority penetrates the tissue in multiple directions, where it is either absorbed by specific components or scattered. Light absorption is closely linked to the chemical composition of the fruit, with pigments such as chlorophylls, carotenoids, and anthocyanins being the primary absorbers in the visible spectrum, whereas water plays a dominant role in absorption within the near-infrared range. As light travels deeper into the tissue, it undergoes modifications due to selective absorption, carrying valuable compositional information (Bureau, 2009).

1.2.6.1.1. Vis-NIR spectroscopy

Vis-NIR spectroscopy (Vis: 380–780 nm; NIR: 780–2500 nm) is a well-established screening method for assessing the chemical and physical characteristics of biological materials. Its effectiveness in analyzing substances like small berry fruits stems from the fact that certain molecular groups within these materials absorb light at specific wavelengths. (Li et al., 2019).

Schaare & Fraser (2000) stated that this optical spectroscopy can operate in three distinct modes - reflectance, transmittance, and interactance - each providing valuable information about the material being analysed (Figure 1.6.).

In reflectance mode, light is directed at the surface of the sample, and the amount of light reflected back is measured, making it ideal for assessing external attributes like color and texture.

In transmittance mode, light passes through the sample, and the amount that emerges on the opposite side is recorded, allowing for the analysis of internal properties, such as moisture content and sugar levels.

Finally, interactance mode measures light that penetrates the material but is scattered before exiting near the point of entry, providing insights into both surface and subsurface characteristics. This hybrid method is particularly useful for evaluating internal structures without needing full light transmission (Ozaki et al., 2006; Wang et al., 2022).

Schaare & Fraser (2000) compared this modality for their ability to non-destructively estimate harvest SSC, density and internal flesh color of the yellow-fleshed kiwifruit (*Actinidia chinensis*) (Figure 1.6.).

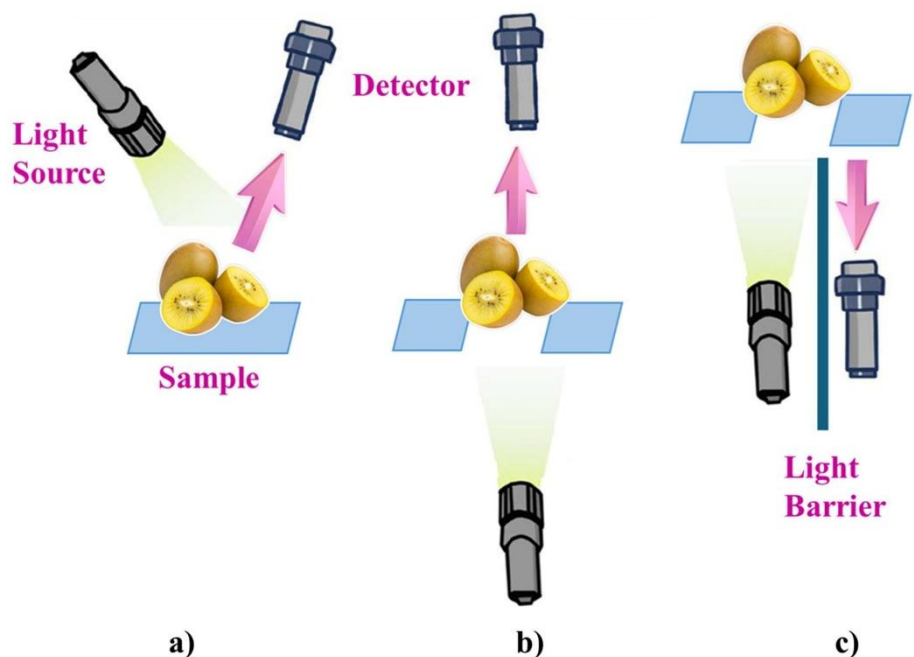


Figure 1.6. Different modes of spectroscopic techniques: (a) Reflectance mode, (b) Transmittance mode, (c) Interactance mode

Interactance mode spectra seem to provide the most precise estimates for parameters such as SSC, density, and flesh color. While interactance mode delivers more accurate results for kiwifruit characteristics compared to reflectance and transmittance modes, achieving proper light sealing can be challenging due to the high conveyor speeds used in modern fruit sorting systems. As a result, reflectance and transmittance modes are more commonly employed in these systems.

In contrast to kiwifruit, better predictions for olive firmness were achieved using transmittance mode over reflectance, resulting in more precise models. This suggests that the choice of spectroscopic mode depends on the specific characteristics of the fruit and the requirements of the analysis system (Kavdir et al., 2009).

NIR spectroscopy, unlike VIS-NIR spectroscopy, focuses exclusively on the near-infrared region of the electromagnetic spectrum, ranging from approximately 750 nm to 2500 nm. In NIR spectroscopy, the sample is exposed to near-infrared light, and either the reflected or transmitted radiation is detected for analysis. As the radiation passes through the sample, its spectral properties are altered due to wavelength-dependent scattering and absorption. These changes are influenced by the chemical composition of the product and its light scattering characteristics, which are linked to the microstructure.

To extract meaningful data from the often complex spectra, advanced multivariate statistical methods, like partial least squares regression, are used (Bellincontro et al., 2011, 2012; Muganu et al., 2013).

NIR spectroscopy was first applied in agricultural settings by Norris (1964) to measure moisture in cereals. Since then, it has been used to analyse the chemical composition of materials, such as water, sugars, moisture, proteins, and lipids in a wide range of agricultural and food products, without the need for complex sample preparations. Early applications in horticulture focused on the dry matter content of onions, SSC content in apples, and water content in mushrooms, but many other applications have followed. As the propagation of NIR radiation through fruit and vegetable tissues is influenced by their microstructure, it was soon discovered that NIR spectroscopy could also be used to measure attributes related to microstructure, such as firmness, internal damage, and even sensory attributes (Nicolai et al., 2007; Norris, 1964).

Non-destructive assessment of food quality using the NIR spectroscopy method has been widely applied for oils (Bellincontro et al., 2012), protein (Fabian & Măntele, 2002), dry matter (Cozzolino et al., 2008), firmness and total soluble solids (Bellincontro et al., 2009). It has been demonstrated that NIR technology shows good prediction capability for bioactive such as anthocyanins and phenols, but also for sugars and titratable acidity. This technology has proven useful to investigate the most significant markers of ripening evolution and quality characteristics of fruit but presents the important advantage that it can be used directly in-field. (Barnaba et al., 2014; Bellincontro et al., 2012; Littarru et al., 2024).

Numerous studies use NIR spectroscopy for the analysis of quality parameters on fruits such as apricots (Camps & Christen, 2009), apple (Clark et al., 2003) kiwifruit (H. Li et al., 2024; Wang et al., 2020), pear (Lu et al., 2022).

Ciccoretti et al. (2019) investigated the use of near-infrared spectroscopy combined with chemometric analysis for the characterization and commercial classification of green-fleshed kiwifruit (*A. deliciosa*). Their findings confirmed that NIR spectroscopy is a reliable, non-destructive, and efficient method for assessing kiwifruit quality in a sustainable manner. The study achieved accurate calibration models for conventional quality parameters, including soluble solids, firmness, titratable acidity, and dry matter. Additionally, strong models were developed for nutritional attributes such as total phenols, carotenoids, and antioxidant activity,

while reasonably good predictions were obtained for flavans and vitamin C (Ciccoritti et al., 2019).

1.3. An overview of kiwi cultivars and their characteristics

Subtropical fruits include a diverse range of species, each offering distinct flavors and traits. Renowned for their high nutrient content, these fruits are rich in vitamins, antioxidants, and essential nutrients, providing both delicious taste and several health benefits to consumers. Often recognized for their vibrant colors, juicy textures, and tropical-inspired flavors, they are a popular choice in a wide variety of dishes, drinks, and desserts. Subtropical fruits, including citrus varieties such as oranges, lemons, and grapefruits, along with kiwifruits, figs, and persimmons, need warm temperatures which are essential for their optimal growth (Rajan et al., 2024).

1.3.1. Kiwi history: a fruitful voyage across continents

Kiwifruit, originally known as the “Chinese gooseberry”, is a small but highly nutritious fruit that evolved from a wild, scarcely utilized plant into a globally significant commercial crop throughout the 20th century. Native to the temperate forests of the mountains and hills of southwest China, where it had been recorded as early as the 12th century, kiwifruit (*Actinidia deliciosa*) was first introduced to the world outside China by adventurous European botanists exploring the region in the 1700s and 1800s. One such explorer Scottish botanist Robert Fortune, was a plant collector, who played a key role in bringing early samples of the kiwifruit vine to Europe. Initially, these vines were introduced as novelty plants in the late 1800s and early 1900s, attracting attention mainly from plant enthusiasts rather than being recognized for their potential as a fruit crop (Ward & Courtney, 2013).

In 1903, New Zealand’s Isabel Fraser encountered the kiwifruit during a visit to China and one year later, she returned to her country with seeds of the kiwifruit. This marked the beginning of kiwifruit cultivation in New Zealand. By the mid-1930s, kiwifruit began to spread beyond the immediate growing regions within New Zealand and in the 1950s the country exported a modest consignment of 2,000 trays of what still known as "Chinese Gooseberries" to London, marking the beginning of the fruit's global commercial presence.

The name "kiwifruit" was awarded by Frieda Caplan, who recognized its marketing potential. She suggested that New Zealand growers adopt this name, inspired by the fruit’s brown, fuzzy exterior which resembled the native kiwi bird New Zealand.

Following her suggestion, Turners & Growers, a leading New Zealand fruit marketing company, officially adopted the new name. It quickly gained widespread acceptance in commercial, scientific, and technical circles solidifying its place as the fruit's internationally recognized identity (Ferguson & Bollard, 1990).

In the aftermath of World War II, combined with agricultural initiatives and marketing strategies from the 1950s onward, kiwifruit orchards quickly spread across various regions, including New Zealand, Australia, Chile, the United States, and several European countries, particularly Italy, France, and Greece. In Italy, the fruit's rich vitamin C content earned it the nickname "*frutto della salute*", meaning the health fruit (Richardson et al., 2018). Kiwifruit have been known in Italy for nearly 90 years, with *Actinidia deliciosa* plants having been listed for sale as ornamental varieties as early as 1934 in the catalog of Giardino Allegra from Catania, Sicily. However, it wasn't until the late 1960s that the first commercial kiwifruit orchards were established in the country.

Experimental plantings of *A. deliciosa* began around 1966-1967 near Lake Maggiore, and over the next two to three years, small demonstration plots appeared in other regions of Italy. These early plantings featured cultivars like 'Hayward' and 'Bruno,' along with selected pollenizers, inspired by several articles published in popular French literature (Testolin, 2015). At the end of the last century, Italy had become the biggest producer of kiwifruit in the northern hemisphere, followed by France, the Iberian Peninsula and Greece, while, in the southern hemisphere, New Zealand and Chile emerged as the leading producers (Costa et al., 2017).

The Food and Agriculture Organization (FAO) of the United Nations provides detailed data on global kiwifruit production. According to the latest available statistics, dating back to 2021, China is the leading producer, with a staggering 2.4 million tons produced. New Zealand follows as a major player, producing around 615.000 tons of the global supply. Italy is also a key player in the global market, producing approximately 320.000 tons of kiwifruit, making it one of the largest producers in Europe. Other significant producers include Greece, Iran, Chile, Turkey, France, Argentina, and Spain (Figure 1.7.), each contributing various amounts to the global supply (Fortin, 2023).

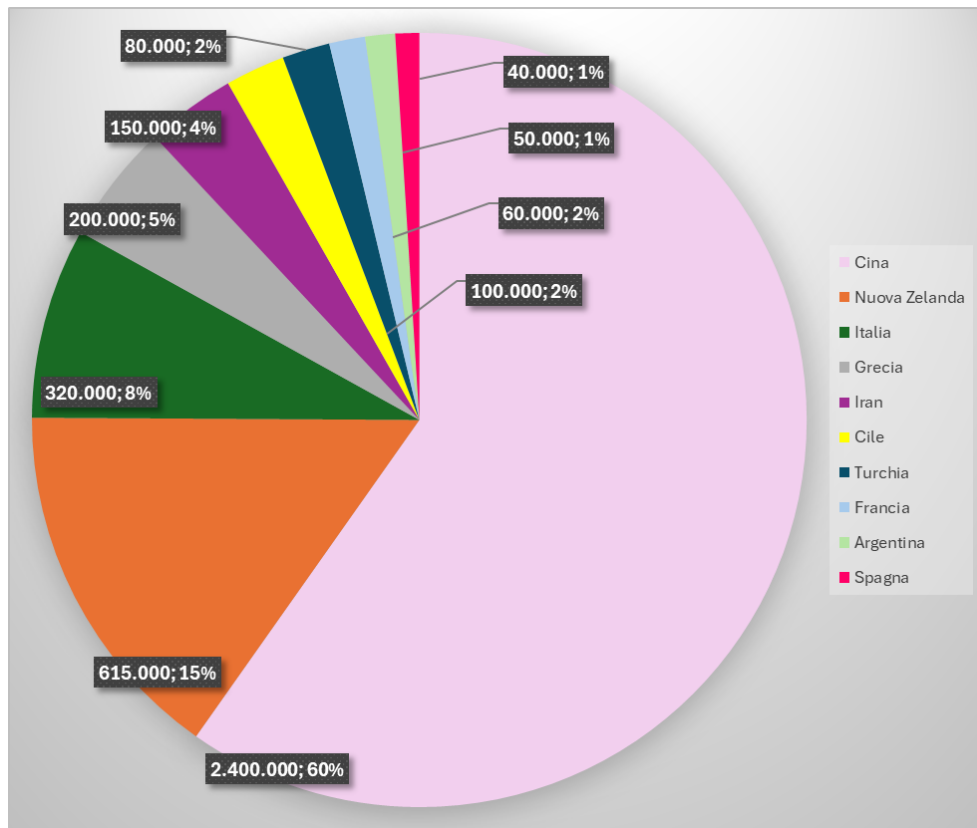


Figure 1.7. Distribution of global kiwifruit production in 2021: the top 10 producing countries and their respective share of global production. Data is presented in tonnes, with China leading the production, followed by New Zealand and Italy. *Source: FAO, 2021.*

1.3.2. The diversity of kiwifruit cultivars

Botanically, kiwifruit is classified as a berry, characterized by thin, smooth skin and a few layers of deep cells beneath the epidermis. Its flesh is divided into three regions: the outer pericarp (OP), the inner pericarp (IP) with seeds (S), and the columella. Each of these regions varies in composition and texture (Guroo et al., 2017). OP, located just beneath the epidermis, consists of thin-walled elliptical parenchyma cells, which are separated from the inner pericarp (IP) by a cylindrical network of vascular bundles. Both the outer and inner pericarp cells contain plastids with chlorophyll. Embedded within the inner pericarp are 20-40 seed locules (carpels) (L), which are slit like chambers running nearly the full length of the fruit. These seed locules contain a mucilaginous matrix that supports the small black seeds. The columella, or white core, lies within the inner pericarp, composed of large parenchyma cells that lack chlorophyll (Figure 1.8.) (Kolahi-Ahari, 2006).

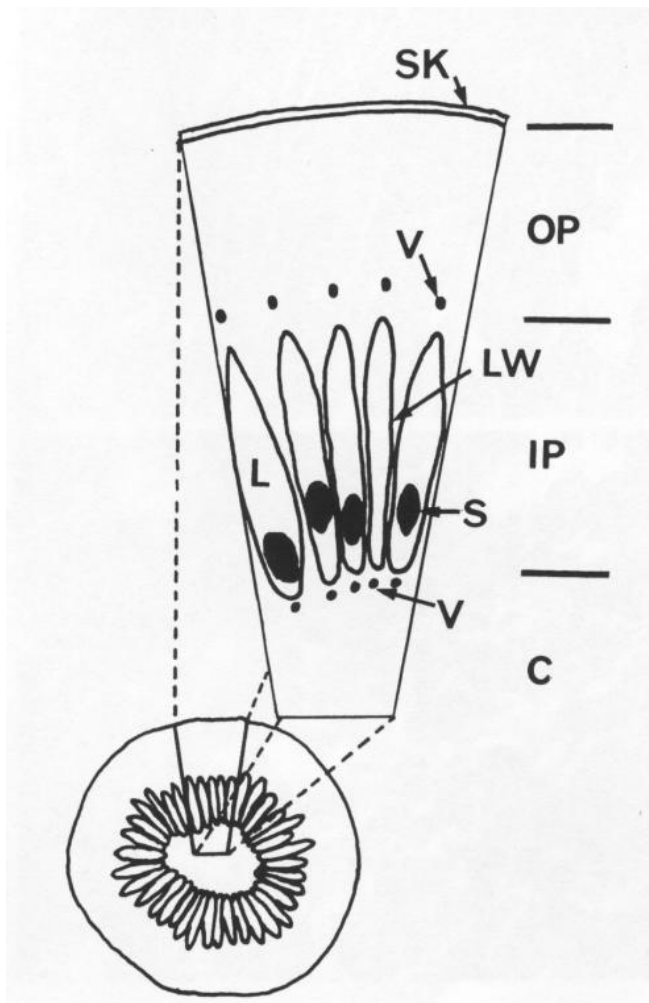


Figure 1.8. Diagrammatic cross-section of a segment of kiwifruit. C= core, IP= inner pericarp, L= locule, LW= locule wall, OP= outer pericarp, S= seed, SK= skin layers (epicarp), V= location of major vascular trace (Hallett et al., 1992)

Kiwifruit belongs to the family *Actinidiaceae* and genus *Actinidia*, which includes 76 species (Guroo et al., 2017). However, among these, the commercial kiwifruit encompasses varieties such as:

- *Actinidia deliciosa*, is the most commercially cultivated species within the *Actinidia* genus, the traditional green-fleshed kiwifruit (Lagos et al., 2017) (Figure 1.9.).
- *Actinidia chinensis*, is another species within the *Actinidia* genus, closely related to *A. deliciosa*, but it is best known for producing the yellow flesh kiwifruit.

This species has become increasingly popular due to its sweeter taste and smooth, hairless skin (Black et al., 2012) (Figure 1.10.).

- *Actinidia arguta*, commonly known as kiwi berry or baby kiwi, is a smaller, more compact species of *Actinidia* that produces small, smooth-skinned fruits similar in taste to the larger kiwi but without the fuzzy exterior (Figure 1.11a) (Medda et al., 2022).

- *Actinidia kolomikta* (Arctic kiwifruit) is a cold-hardy climbing plant prized for both its ornamental beauty and small, sweet kiwi-like fruits. Its variegated leaves, ranging from green to pink and white, make it highly decorative, while its frost resistance makes it ideal for colder climates (Figure 1.11b) (Zuo et al., 2012).
- *Actinidia purpurea* (purple kiwifruit) is a lesser-known kiwi species native to China and Vietnam. It produces small, round fruits with smooth, edible skin and vibrant purple or pink flesh. This cold-hardy, deciduous vine is valued for both its unique fruit and ornamental appeal, making it an interesting addition to gardens in temperate climates (Figure 1.11c) (Huang, 2016).
- *Actinidia polygama* (silver vine) is a deciduous climbing plant native to East Asia. It is famous for its striking silvery leaves and the fragrant, sweet-smelling flowers it produces. Unlike other kiwis, its small fruits are not typically eaten, but the plant is often grown for ornamental purposes (Figure 1.11d) (Motohashi, 1991).
- *Actinidia eriantha* (Velvet vine) also known as the white-fleshed kiwi, is a species native to China. It produces small, oval fruits with smooth skin and sweet, white flesh. While not as widely known as the traditional green kiwi, it is prized for its unique flavor and smooth texture (Figure 1.11e) (Huang, 2016).
- *Actinidia melanandra* (red kiwifruit) is a lesser-known species of kiwi native to China. It is characterized by its small, round fruits with dark purple skin and a sweet, slightly tart flavor. It is a hardy vine, adaptable to temperate climates (Figure 1.11f) (Chesoniene et al., 2004).

Among these species, *Actinidia chinensis* var. *deliciosa*, commonly known as fuzzy kiwifruit, is particularly notable for its larger fruit size, higher yield, lower respiration rate, and reduced ethylene sensitivity, resulting in the longest shelf life. The fruit is characterized by its fuzzy, dull-brown skin, while the inside reveals vibrant green flesh (Figure 1.9.) (Guroo et al., 2017).

Among the numerous *A. deliciosa* cultivars, ‘Hayward’ is the most extensively cultivated, accounting for approximately 90% of global kiwifruit production. From a market standpoint, its fruits are highly appealing to consumers due to their vibrant, translucent green flesh and excellent shelf life. Additionally, ‘Hayward’ is widely grown across various regions due to its remarkable yield, larger fruit size, and elevated soluble solids content compared to other varieties (Y. F. Li et al., 2021).



Figure 1.9. *Actinidia chinensis* var. *deliciosa*

On the other hand, *Actinidia chinensis* var. *chinensis*, also known as the smooth-skinned kiwifruit, is distinguished by its smooth, bronze-colored skin and unique beak-like shape at the stem attachment. The flesh can vary from bright green to a vibrant golden hue, and it is renowned for being sweeter and more aromatic than its green-fleshed counterparts. This variety commands a premium price in the market due to its smoother texture and enhanced flavor profile. However, its shorter storage life limits its widespread commercialization compared to *Actinidia deliciosa* (Guroo et al., 2017).

Actinidia chinensis ‘Soreli’, the yellow-fleshed kiwifruit, is a highly regarded fruit variety known for its excellent balance of sugar and acidity, as well as its high ascorbic acid content (Goffi et al., 2020). This variety is particularly valued for its early ripening, medium to large fruit size, and exceptional quality and nutritional value. However, despite these advantages, its storage lifespan in commercial refrigeration is shorter compared to green-fleshed kiwifruit varieties like ‘Hayward’ (Goffi et al., 2021; Grasso et al., 2022).

A. chinensis ‘Hongyang’ is a nearly hairless cultivar with smooth skin and is recognized as the first commercially available red-fleshed variety. It has a higher sugar-to-acid ratio than many other cultivars, resulting in an enhanced flavor that appeals to consumers. Additionally, its vitamin C content *exceeds* that of ‘Hayward’. However, its fruits are comparatively smaller, and the plants *are more susceptible* to bacterial canker, leading to a higher market price than ‘Hayward’ (Li et al., 2021; Ma et al., 2017).



Figure 1.10. *Actinidia chinensis* var. *chinensis*

Hort16A (*Actinidia chinensis* Planch. var. *chinensis*), belonging to *Actinidia chinensis*, is a particularly striking cultivar of this species displays a red halo encircling the center, contrasting with its yellow pulp. This cultivar, developed in New Zealand, is characterized by an oval shape with a prominent distal end, unlike the 'Hayward' variety, which has a rounded tip. Its skin is smooth, thin, and bronze-colored, while the flesh ranges from bright green to intense yellow, offering a sweeter and more aromatic flavor compared to green kiwifruit. It was once widely distributed but saw a decline in availability after suffering extensive damage in New Zealand from the PSA bacterium between late 2010 and 2013 (Boyd et al., 2008; Burdon et al., 2002).

The berries of *Actinidia arguta* undergo rapid softening, followed by significant moisture loss during storage. In contrast, *Actinidia melanandra*, commonly known as red kiwifruit, is a dioecious climbing plant that produces clusters of large, reddish-green, grape-like fruits. These berries are notably sweeter and more intensely flavored than *Actinidia deliciosa* and possess a smooth texture, allowing them to be eaten whole. This highly productive species is remarkably winter-hardy, withstanding temperatures as low as -20°C . Rich in bioactive compounds, *A. melanandra* berries exhibit exceptionally high antioxidant activity. However, despite their nutritional benefits, this species has limited shelf life presents a challenge for large-scale commercial cultivation (Kaya et al., 2016).



Figure 1.11. Different varieties of *Actinidia chinensis*. a. *Actinidia arguta* b. *Actinidia kolomikta*. c. *Actinidia purpurea*. d. *Actinidia polygama*. e. *Actinidia eriantha*. f. *Actinidia melanandra*

1.3.3. Qualitative Features of Kiwifruit

Kiwi plant yields can vary from 30 to 60 t/ha according to the cultivar, training and pruning and the use of plant growth regulators (Testolin, 2015). Its fruits were described as the size of a walnut with the flavor of ripe gooseberries, offering a distinctive tangy-sweet taste. The chemical composition of kiwifruit plays a crucial role in understanding its nutritional value and associated health benefits. The composition of kiwifruit is influenced by various factors, including horticultural practices, the growing region and soil type, storage and ripening conditions, and, most significantly, the fruit's maturity at harvest. Traditionally, kiwifruit has been valued for its nutritive components, such as proteins, lipids, carbohydrates, vitamins, and minerals, and is widely recognized as a rich dietary source of vitamin C, potassium, folate, and fiber. However, to date there is a growing interest in the antioxidant capacity, enzyme activity, and polyphenolic and phytochemical content of kiwifruit (Drummond, 2013). This focus is reflected in the increasing volume of scientific publications exploring these aspects, highlighting the fruit's potential in contributing to health beyond its basic nutritional profile (Drummond, 2013; Gullo et al., 2016; Kaur et al., 2022; Kaur & Kapoor, 2001; Zhu et al., 2008).

1.3.3.1. Ascorbic Acid (AA)

Vitamin C (total ascorbic acid) content is the most important and distinctive nutritional attribute of kiwifruit. Within species, reported AA levels vary considerably, and this is subject to further variation within cultivars. In Hayward cultivar, AA levels typically range from 80 to 120 mg per 100 g of FW (U.S. Department of Agriculture, 2011), while yellow-fleshed varieties contain higher values, up to 220 mg per 100 g of FW (I. Nishiyama et al., 2004). These values can vary based on factors such as growing region, environmental conditions, fertilization, harvest maturity, timing of harvest, storage, and ripening conditions. In terms of nutritional value, kiwifruit rank among the top fruits, primarily due to their exceptional vitamin C content (Figure 1.12.), as indicated by scoring models that assess and compare essential nutrients. Yellow kiwifruit, for example, contains 161.3 mg of vitamin C per 100 g, nearly three times the amount found in oranges and strawberries on an edible flesh weight basis (Richardson et al., 2018).

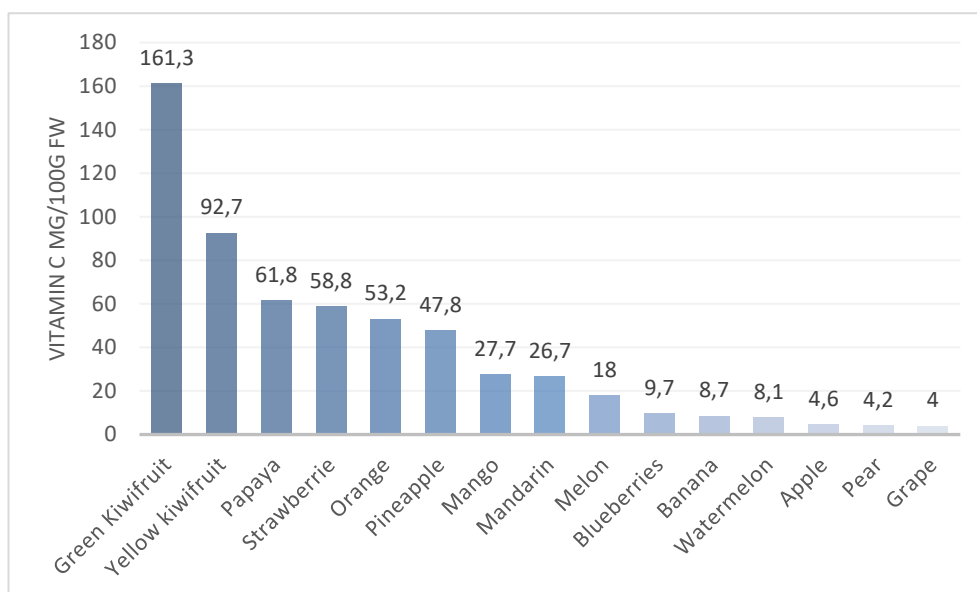


Figure 1.12. Graph comparing the vitamin C content of kiwifruit with other commonly consumed fruits. Source: Richardson et al. (2018)

Vitamin C offers numerous health benefits for humans, playing a crucial role in supporting the immune system, helping the body fight off infections and illnesses. Additionally, it acts as a powerful antioxidant, protecting cells from damage caused by free radicals. Vitamin C is essential to produce collagen, a protein that supports skin, blood vessels, and bones, contributing to overall skin health and wound healing. Moreover, adequate vitamin C intake has been linked to improved iron absorption and a reduced risk of chronic diseases (Oz, 2010; Tavarini et al., 2008).

1.3.3.2. Vitamin E

Kiwifruit flesh contains a high content of Vitamin E compared to other fruits (1.4 - 1.5 mg per 100 g of fruit). For many years, it was assumed that the vitamin E of kiwifruit was contained predominantly in the seeds in association with the oil content, and not bioavailable as the seeds typically resist digestion (Ferguson A. R. & Ferguson L. R., 2003). However, more recent analysis has shown that the main α -tocopherol form of vitamin E is in the flesh, possibly associated with cell membranes, and therefore potentially bioavailable (Kaur et al., 2022).

A new form of vitamin E in kiwifruit, d-tocomonoenol, was recently studied and found to have high antioxidant capacity. Higher levels of d-tocopherol and d-tocomonoenol were recorded in the skin compared to the flesh, compared with similar levels of α -tocopherol in both tissues (Drummond, 2013).

1.3.3.3. Potassium

Both green and yellow kiwifruits are rich in potassium, with approximately 301–316 mg per 100 g. These values meet the criteria set by EU Regulation (EC) no. 1924/2006 for making a natural 'source' claim, as they exceed 15% of the daily Reference Intake of 2000 mg (Drummond, 2013; Richardson et al., 2018).

The potassium in kiwifruit offers numerous health benefits. It helps regulate blood pressure by balancing sodium levels, thereby reducing the risk of hypertension and cardiovascular diseases. Additionally, potassium supports muscle and nerve function, preventing cramps and ensuring proper muscle contractions, including those of the heart (Curhan, 1997; Gennari, 1998).

1.3.3.4. Dietary fibre

Kiwifruit's dietary fibre primarily comes from its plant cell walls, especially the polysaccharides that form the key structural components of these walls. Around 2–3% of the fresh weight of kiwifruit consists of non-starch polysaccharides, which contribute valuable soluble and insoluble fibre to the diet (Lassner & Shubert, 2006). Studies on both green and gold kiwifruit show that they contain approximately one-third soluble fibre and two-thirds insoluble fibre, with gold kiwifruit having a lower total fibre content than green. The soluble fibre is mainly composed of pectic polysaccharides, while the insoluble fibre is predominantly made up of cellulose and hemicelluloses (Drummond, 2013; Richardson et al., 2018).

1.3.3.5. Sugars

As kiwifruit develop and ripen, the concentrations of various chemical components in the tissue undergo significant changes. The level of soluble sugars present in kiwifruit is one of the key indices of ripeness, as during the maturation and ripening process, the concentration of sugars increases significantly concomitant with a reduction in starch content and changes in fruit texture (Drummond, 2013).

In ripe kiwifruit, the natural sugars contribute to its pleasant sweetness, which is complemented by its acidic components for a well-balanced flavor (I. Nishiyama et al., 2004). The predominant sugars present in *Actinidia* are glucose, fructose, and sucrose. The amount of total sugars and ratios of those sugars vary not only as a function of maturity but also with the variety of kiwifruit (Luh & Wang, 1984; Nishiyama et al., 2008).

When fruit is mature but not fully ripened, it can be harvested and will continue to ripen successfully after being picked. By storing the fruit in cool conditions immediately after harvest, the ripening process slows down. This ability to control the ripening process is what allows kiwifruit to be shipped to distant markets over an extended period. To ensure optimal eating quality, suitable maturity indicators are used, considering factors such as flesh firmness, starch conversion to sugars, and soluble solids concentration. These indicators help determine when kiwifruit is at the ideal stage for harvest (D. P. Richardson et al., 2018).

1.3.3.6. Antioxidants

In addition to the well-known nutrients like vitamins C and E, kiwifruit also contains a variety of minor compounds that may contribute to beneficial physiological effects. These include antioxidants such as lutein (I. Nishiyama et al., 2007), zeaxanthin (Xia et al., 2021), β -carotene (I. Nishiyama et al., 2007; Xia et al., 2021), chlorophylls (I. Nishiyama et al., 2007), quinic acid (Marsh et al., 2009), caffeic acid glucosyl derivatives (C. Zhu et al., 2021), β -sitosterol (Fiorentino et al., 2009), chlorogenic acid (C. Zhu et al., 2021), and phenolics, including flavones and flavonones (Fiorentino et al., 2009; Tavarini et al., 2008; C. Zhu et al., 2021). The total antioxidant capacity of kiwifruit has been reported to surpass that of apple, grapefruit, and pear, although it is lower than that of raspberry, strawberry, orange, and plum (Drummond, 2013). Several human studies have linked the antioxidant compounds in kiwifruit to positive changes associated with health benefits.

Free radicals in the human body contribute to oxidative damage affecting molecules like lipids, proteins, and nucleic acids. This oxidative stress is linked to the onset of several degenerative diseases. Therefore, antioxidants that can neutralize these free radicals (ROS) may significantly aid in preventing conditions such as cancer, cataracts, neurological disorders, and rheumatoid arthritis (García-Alonso et al., 2004).

Furthermore, the production of ROS as superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) is considered an important event in fruits during storage. While ROS serve as signaling molecules and secondary messengers in the activation of stress and defense mechanisms, their excessive buildup can result in oxidative damage to biomolecules (S. Zhu et al., 2008). Various enzymes are involved in the process of scavenging ROS in kiwifruit, including superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and peroxidase (POD; EC 1.11.1.7). Within a cell, SODs constitute the first line of defense against ROS. SOD are metalloproteins belonging to a class of oxidoreductase 1 enzymes. This critical enzyme is responsible for elimination of superoxide radicals and is considered to be a key antioxidant in aerobic cells, because its activity catalyses the dismutation of superoxide to H_2O_2 and oxygen, and H_2O_2 is further detoxified by CAT and POD (Goffi et al., 2020; Kaur & Kapoor, 2001; W. Lu et al., 2021; Meitha et al., 2020; S. Zhu et al., 2008). CAT is a crucial antioxidant enzyme that decomposes H_2O_2 , a toxic by-product of cellular metabolism, into water and oxygen. This process is essential for protecting the fruit's cells from oxidative damage. POD is another key antioxidant enzyme that uses H_2O_2 to oxidize various organic substrates such as phenols and aromatic amines, critical in defense mechanisms. So, CAT and POD work in tandem to regulate H_2O_2 levels and maintain the redox balance within the fruit cells. While catalase rapidly removes hydrogen peroxide, peroxidase utilizes it in biochemical reactions that enhance the fruit's structural defenses (Gill & Tuteja, 2010; Li et al., 2021).

1.3.4. Climate change impacts on kiwifruit cultivation systems

Climate change poses significant challenges to agricultural systems worldwide. These changes may affect the adaptability of temperate fruit trees that account for about 48% of global fruit production. Among the myriad impacts of climate change, the cultivation of kiwifruit trees (*Actinidia* spp.) faces multifaceted challenges. (Rajan et al., 2024).

One of the most critical factors is the increase in air temperature, which has been linked to the spread of ‘vine decline syndrome’.

In regions like Italy, rising summer temperatures have accelerated the spread of this syndrome, a complex condition that can be triggered by multiple factors including water pollution, soil pathogens, contaminants, and prolonged waterlogging. Once established, the syndrome can progress rapidly, leading to severe plant stress and eventual death (Bardi, 2020; Donati et al., 2020).

Elevated temperatures also result in decreased oxygen availability in the soil, which alters root metabolism and hampers their growth. Instead of healthy, deep root systems, plants develop fibrous roots that are less efficient in nutrient and water uptake. This impaired root function not only weakens the plant but also reduces its resilience to environmental stressors (Bardi et al., 2020). Furthermore, increased air temperatures promote excessive vegetative growth at the expense of fruit development. This shift in resource allocation diminishes carbohydrate accumulation in the fruit, leading to lower sugar content and poorer quality. Additionally, it disrupts the plant’s energy balance, reducing flower formation in the subsequent season and ultimately affecting future yields (A. C. Richardson et al., 2004).

Another significant challenge is waterlogging, often exacerbated by erratic rainfall patterns and extreme weather events associated with climate change. Kiwifruit plants are highly sensitive to saturated soil conditions, which can weaken their physiological functions and make them more susceptible to attacks from pathogenic microorganisms. Persistent waterlogging not only stresses the plant but also fosters the development of diseases that accelerate plant decline (Savian et al., 2020).

Nutrient dynamics in kiwifruit are also influenced by climatic variations. The concentrations of phosphorus, nitrogen, magnesium, and sodium in certain kiwifruit cultivars have been shown to correlate with precipitation levels during early phenological stages. For instance, higher rainfall during these phases can affect nutrient uptake, while sodium content in particular has been found to correlate negatively with elevated temperatures during flowering and fruit set. These nutrient imbalances can impair fruit development, leading to suboptimal size, taste, and nutritional value (Donati et al., 2020; Savian et al., 2020).

In summary, the combined effects of increased temperatures and waterlogging due to climate change pose significant threats to kiwifruit cultivation.

They not only disrupt physiological processes such as root metabolism and nutrient absorption but also reduce fruit yield and quality, posing challenges for sustainable production in traditional growing regions (Bardi, 2020; Donati et al., 2020).

1.4. Research objectives and work plan

This research project was conducted at Tre Colli S.r.l., located in Cisterna di Campoleone (Velletri, Rome, Italy), a renowned producer of kiwi fruits (Figure 1.13.), not only the traditional green variety, but also the more recent yellow flesh variety and a new cultivar with red flesh, both of which are the focus of our research project. In recent years, the company has been progressively expanding its product range, also embracing the production of organic pomegranates and lemons. Moreover, in recent years, the company has been incorporating advanced technologies and novel treatments within the agri-food supply chain, aiming to enhance competitiveness, production efficiency, quality, and sustainability.



Figure 1.13. Fields of ‘Soreli’ kiwi plants at Tre Colli S.r.l. (Cisterna di Campoleone, Velletri, Rome, Italy) on which the experimentation was carried out in the 2022-2023 and 2023-2024 years

Our efforts focused on both yellow kiwi cultivars (*Actinidia chinensis* 'Soreli') and a newly developed red kiwi cultivar, which is currently awaiting its official designation.

For yellow kiwifruit variety ‘Soreli’, our study focused on tackling key environmental challenges by optimizing resource use to curb pollution and limit water wastage—factors that have become central in contemporary agri-food sustainability debates. The goal was to decrease irrigation water usage without compromising, and ideally even enhancing, fruit quality and plant health.

To achieve this, we integrated real-time data collection from IoT-based sensors, known as "Tree Talkers," developed by Nature 4.0 S.r.l. (Viterbo, Italy). These sensors allow to monitor precise, real-time data directly from the plants, offering numerous advantages. Specifically, this technology provides optimized water usage by precisely measuring plant needs, reducing resource waste, and fostering a more sustainable agricultural practice. Additionally, real-time insights from these sensors facilitate timely interventions, preventing issues related to plant health and contributing to the consistent quality of the fruits (Valentini et al., 2019). Furthermore, comprehensive post-harvest analyses - including technological, chemical, enzymatic, and antioxidant evaluations - were performed on these kiwis to assess fruit quality thoroughly. This multi-faceted analysis allowed us to understand the impact of our optimized irrigation strategies on fruit properties, ensuring that reduced water use did not compromise, and potentially even improved, the nutritional and market quality of the kiwifruits.

Furthermore, the company aimed to evaluate the post-harvest preservation of the new red kiwi cultivar and investigate techniques to intensify its red pigmentation, which was primarily concentrated in the central pulp without extending into the outer flesh. To improve these qualitative aspects, we treated the red kiwi plants with a ripening inducer, *cis*-3-hexenyl butyrate (Payá et al., 2020) to evaluate its potential effects on various ripening characteristics, including color intensity, sugar content, and anthocyanin concentration. To validate these findings, we employed AOTF-NIR (Acousto-Optic Tunable Filter Near-Infrared Spectroscopy), a non-destructive method that provided substantial benefits to our analysis. This method allowed us to monitor fruit characteristics in real time without damaging the samples, thus preserving the fruit for further tests and reducing the need for invasive sampling. Additionally, AOTF-NIR provided high precision and reproducibility in measuring key quality attributes, facilitating a more comprehensive and efficient evaluation of the effects of the ripening inducer on the red kiwi cultivar.

Establishing a digital, high-quality, and health-focused supply chain is essential for agri-food companies to ensure transparency and traceability in horticultural products, offering consumers guarantees of food safety and certified quality. Digitalization enables precise monitoring at each stage of the production process, from cultivation through distribution, minimizing waste and increasing operational efficiency.

Furthermore, a health-focused supply chain is crucial for meeting high sanitary standards and addressing the growing market and consumer demand for sustainability and product provenance. Investing in a quality-driven supply chain allows agri-food companies to enhance the value of their products, strengthen consumer trust, and improve market competitiveness. Ultimately, this project aims to increase the effectiveness and efficiency of food traceability processes, deliver substantial benefits to the supply chain, boost sales, and harness data as a strategic knowledge asset for businesses.

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CHAPTER II

This project, published as an article in *Chemical Engineering Transactions* 102 (2023) and presented at 3th International conference on engineering future food, presents the results from the first year (2022-2023) of my PhD research project. The focus of the study was on *Actinidia chinensis* 'Soreli' kiwifruit plants and their responses to different irrigation treatments. To assess plant and leaf health, we employed IoT devices known as TreeTalkers, which provided real-time monitoring of physiological parameters. Additionally, we conducted chemometric and technological analyses on the harvested fruits to evaluate their quality and composition. The data collected and the results discussed offer valuable insights into the effects of irrigation management on both plant well-being and fruit characteristics. These findings are detailed in the following sections.

2. Real-time Continuous IoT-TT Spectrum as a Predictor of Plant Health and Fruit Quality in 'Soreli' kiwifruit

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2.1. Introduction

Kiwifruit is appreciated by consumers for its high content of bioactive compounds, which have health-beneficial effects. Consequently, the consumption of fresh kiwifruits is booming (Meena, Nirmal Kumar, et al. 2018). Yellow-fleshed kiwifruit (*Actinidia chinensis* 'Soreli'), known for its sweeter flavor, possesses numerous agronomic features, including early ripening, high productivity, and large fruit size with excellent taste and nutritional quality traits (Goffi, Magri, et al. 2020). However, this fruit is not suitable for long-term storage (Goffi, Modesti et al. 2018). The lack of proper maturity harvest indices poses a challenge in postharvest management, and several factors contribute to the deterioration of fresh fruit quality and storage life. Therefore, developing novel techniques to maintain fruit quality

and prolong shelf life after harvesting is of utmost importance (Gwanpua, Jabbar et al. 2018). It has been observed that inefficient water use increases the cost of crop production, negatively affects the environment and the final product, and can induce changes in fruit yield and quality (Pinto, Valin et al. 2021). Moreover, reducing wastage benefits farmers, consumers, and society as a whole. In fact, kiwifruit plants have a fleshy, shallow root system that is sensitive to waterlogging stress, resulting in decreased crop yield or even plant death. Approximately 16% of cultivated land worldwide is affected by waterlogging, causing an estimated 20% decrease in yield (Liu et al. 2022). It has been established that a slight lack of water can benefit the post-harvest storage characteristics of the fruit (Reid et al. 1996). The technical approach to optimize irrigation programs is based on water moisture content and plant water status (Steduto et al. 2012), to check the plant's health and hydration status. The Internet of Things (IoT) can enhance agricultural and farming industry processes through automation, which involves collecting data using sensors and processing it using controllers (Hari Ram et al. 2015). The current use of smart agricultural systems primarily focuses on water management, crop management, and smart farming, aiming to automate processes by reducing human intervention, costs, power consumption, and water usage (hari Ram et al. 2015).

2.2. Material and Methods

2.2.1. Experimental design

Three experimental plots of 'Soreli' kiwifruit vineyards at the "Tre Colli" farm, located in Cisterna Campoleone (Velletri, Rome, Italy), were selected to study the impact of water deficit on plant health and the quality of the final product. Each of the three plots is characterized by the same mineral composition and cultivation practices. They are subjected to distinct irrigation treatments: default drip irrigation at a rate of 50-100 L per day, depending on seasonal and climatic conditions (referred to as 100% or full irrigation), and deficit irrigation at 80% and 60% of the full irrigation approach. Each experimental plot covers an area of approximately 1 ha of soil surface and contains around 700 vines per hectare, with plants spaced 4.0 m apart between rows and 3.0 m apart within rows.

2.2.2. TT Spectrum and soil sensors

To evaluate the effect of different irrigation methods on plants, three low-cost IoT-TT Spectrums and three soil sensors were used from June to September 2022. Each treatment employed these devices to obtain real-time observations of physical and biological parameters of the leaves and soil moisture. The nine spectrophotometers were placed on support poles of kiwifruit plants, projecting onto the foliage in a diagonal orientation. The selection of these devices was based on their field position and similar exposure to natural diurnal light. The IoT-TT Spectrum is specifically designed to measure canopy reflectance radiance across 12 spectral bands, including six in the visible range and six in the near-infrared range (Figure 2.1.). The soil sensors, positioned horizontally at a depth of 30 cm, provide data on soil volumetric water content (Soil VWC %) for each individual tree. These soil sensors were provided by Nature 4.0 S.r.l (Viterbo, VT, Italy). The data collected is saved in the Cloud and can be accessed through portable software such as tablets, PCs, or phones. The soil sensors and spectrometers were used to collect data on soil water availability and vegetation indices.

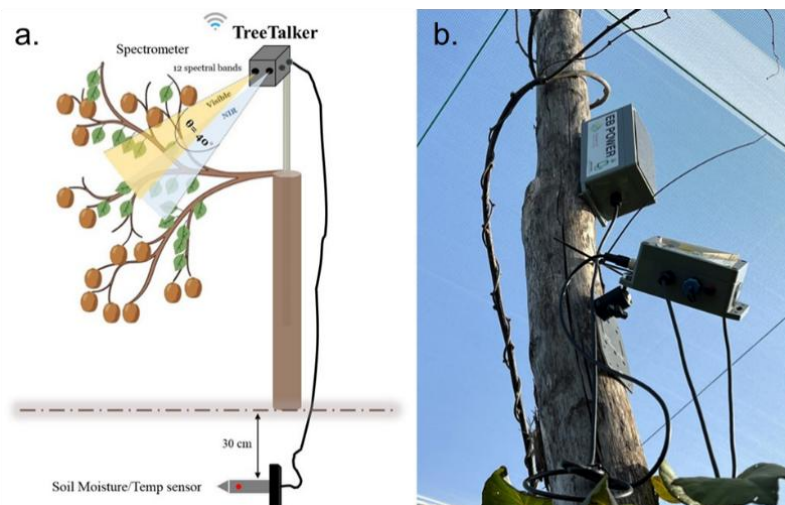


Figure 2.1. a. Schematic of TT-Spectrum measuring light reflectance data in 12 bands, b. Field installation of TT-Spectrum in 'Soreli' kiwi plants at Tre Colli S.r.l. (Cisterna di Campoleone)

TT Spectrum collects light reflectance data in 12 spectral bands, 610+, 680+, 730+, 760+, 810+, 860+ nm, 450*, 500*, 550*, 570*, 600*, 650* (* ± 20 nm + ± 40 nm). The reported data are digital numbers. Before converting the raw data to the physical units, basic quality assurance procedures are applied (i.e., outliers' removal and basic gap filling). Then the data elaboration followed by the conversions from digital numbers to physical units, which are conducted according to the

TreeTalkers' user manual (User Manual-string type 59 and 49, March 2023). For further processing, four vegetation indices, Normalized Difference Vegetation Index (NDVI) (Tucker, 1979), Chlorophyll Vegetation Index (CVI) (Vincini et al. 2008), Leaf Nitrogen Balance Index (LNBI) (Fan et al. 2022) and Anthocyanin Index (AI) (Gitelson et al. 2001) are evaluated from TT Spectrum data.

The Normalized Difference Vegetation Index (NDVI) is obtained following the below formula (1):

$$NDVI = \frac{NIR\ 810 - RED\ 650}{NIR\ 810 + RED\ 650} \quad (1)$$

Where:

NIR = reflectivity in the near-infrared spectrum (Band 810)

RED = reflectivity in the red of the spectrum (Band 650)

The Chlorophyll Vegetation Index (CVI) is estimated following the below equation (2):

$$CVI = (NIR\ 810 - \frac{RED\ 650}{GREEN\ 550^2}) \quad (2)$$

Where:

NIR = reflectivity in the near-infrared spectrum (Band 810)

RED = reflectivity in the red of the spectrum (Band 650)

Green= reflectivity in the green of the spectrum (Band 550)

Anthocyanin index (AI) is a vegetation index that has been developed based on the reflectance measurements at 550 nm and 700 nm (3):

$$AI = \frac{(R550 - R700)}{(R550 + R700)} \quad (3)$$

where R550 and R700 are the reflectance values at 550 nm and 700 nm, respectively.

The Leaf Nitrogen Balance Index (LNBI) is a vegetation index that is used as an indicator of nitrogen content in leaves. The spectrometer band that is most sensitive to LNBI is typically in the range of 700-800 nm, which is in the red edge part of the spectrum.

The applied formula for LNBI is (4):

$$LNBI = \frac{(R750 - R705)}{(R750 + R705)} \quad (4)$$

where R705 and R750 are the reflectance values at 705 nm and 750 nm, respectively.

2.2.3. *Kiwi fruit storage conditions*

‘Soreli’ kiwifruits were collected in September 2022 with a °Brix value of 6.8-7.4, ensuring uniform size, appearance, and the absence of defects and disease. These fruits were packed in single-layer trays and promptly transported to the Postharvest Laboratory of DIBAF (University of Tuscia, Viterbo, Italy). The kiwifruits were stored at room temperature for 24 hours and then cooled to 1 ± 0.5 °C with a Relative Humidity (RH) of $85 \pm 5\%$ in a normal atmosphere, utilizing an ethylene absorber. All analyses were performed at harvest time and every 15 days of cold storage, including 0, 15, 30, 45, 60, and 75 days.

2.2.4. *Physical-Chemical Parameters*

At harvest (0 d) and before storage, the same 20 kiwifruits were weighed using a digital balance (Adam Equipment Co., Ltd., Milton Keynes, UK) at each sampling time to monitor weight loss during cold storage. The total soluble solid content (SCC) of the fresh kiwifruit juice was measured using a digital refractometer (ATAGO, Palette PR-32, Tokyo, Japan) and expressed as °Brix (%). Flesh color was evaluated on peeled fruits using a Minolta colorimeter (Minolta C2500; Konica Minolta, Ramsey, NY, USA) to assess the chromaticity values L* (Lightness), a* (green to red), and b* (blue to yellow). Chroma (C*) values were calculated as reported by McGuire (1992). Flesh firmness was assessed in two ways: firstly, with a destructive method by removing a 1 mm thick disc of skin from the equatorial section of each fruit and using a digital penetrometer (Mod. 53205; TR Turoni snc, Forlì, Italy). The results were expressed as N m⁻². Secondly, a non-destructive method was employed using an Instron Universal Tasting Machine (Mod. 4301 Instron Inc., Canton, MS, USA). The fruits (20) were compressed at a speed of 25 mm min⁻¹ and a load deformation of 5 N. The same twenty fruits were used at each

sampling time and were numbered beforehand to ensure the analysis was performed on the same fruits throughout the entire duration of the test. The fruits were rewarmed to ambient temperature before conducting the Instron test and were then returned to the cold rooms afterward. Flesh hardness was expressed in N m^{-2} . The titratable acidity (TA) was measured in triplicate on the flesh juice obtained from five fruits following the protocol of Grasso et al. (2022).

2.2.5. *Bioactive Compounds Content*

Total phenols (TP), expressed as milligrams of gallic acid equivalent per 100 g FW, and flavonoids (TF), expressed as milligrams of catechin equivalent per 100 g FW, were analysed following the protocol of Grasso et al. (2022). The content of ascorbic acid (AA) was assessed according to Grasso et al. (2022), and it was expressed as milligrams of AA per 100 g FW. Chlorophyll a, chlorophyll b, and β -Carotene in the sample were analysed as described by Goffi et al. (2017). Pigments were extracted using acetone-hexane (v:v 4:6), and the optical density of the supernatant was measured at 663 nm, 645 nm, 505 nm, and 453 nm using a spectrophotometer (Perkin Elmer Instruments Ltd., Seer Green, Beaconsfield, U.K.). All the analyses were performed in triplicate.

2.2.6. Statistical analysis

All results are expressed as the means $\pm$ standard deviation (SD). Statistical significances between different maturity stages of the fruit of different orchards and storage time were analysed by two-way analysis of variance (ANOVA), and Tukey's test at 5% level was calculated to compare differences between means. Differences were deemed significant at a p-value of < 0.05 and are denoted by distinct letters.

2.3. Results and Discussion

2.3.1. Effects of Overwatering or Underwatering on Kiwifruit Plants

Soil sensors and spectrometers were utilized to collect data on soil water availability and vegetation indices during the days of the year 2022. We then correlated these two factors for each individual plant to better understand the relationship between soil moisture and plant health. By examining these correlations, we can gain insights into the optimal soil moisture conditions for promoting growth and overall plant health in the ecosystem. Adequate soil moisture is crucial for optimal nutrient uptake and root development in trees, but overwatering or underwatering can be detrimental. Therefore, we collected soil volumetric water content (Soil VWC %) data for each individual tree (Figure 2.2.).

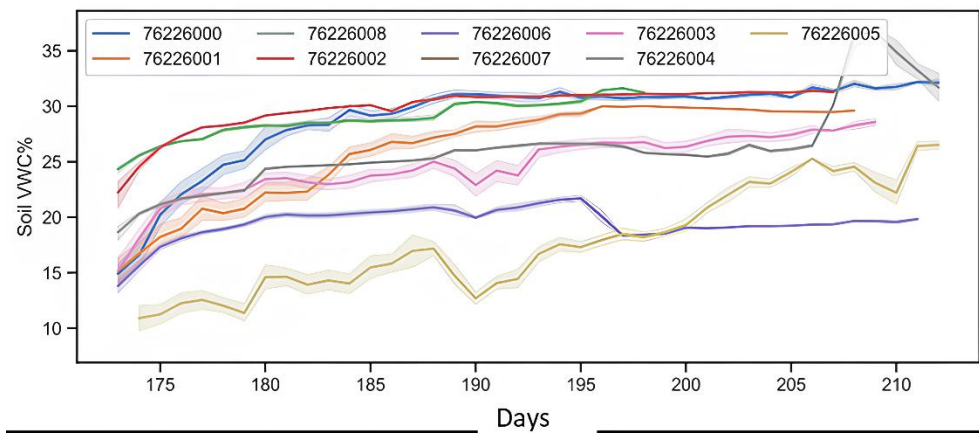


Figure 2.2. For each individual tree, the soil volumetric water content (Soil VWC %) data were collected using soil sensors placed at a depth of 30 cm. (The data for the sensor TT6226007 is missing)

In Figure 2.3. we present the TT-Spectrum output for the main vegetation indices (NDVI, CVI, LNBI, and AI) for each individual, based on the reflectance data from various reported bands (450 to 860 nm). The results demonstrate that different levels of soil moisture availability for kiwifruit plants (Figure 2.2.) lead to distinct ecophysiological responses (Figure 2.3.). The analysed vegetation indices (NDVI, CVI, LNBI, and AI) based on TT Spectrum data provide valuable insights into the ecophysiological responses of kiwifruit plants to different soil moisture levels. The soil moisture data revealed that each individual plant received different levels of water availability, resulting in varying health, growth, and productivity conditions. For example, Tree 76226005 received lower irrigation during the early season and

maximum water availability during the dry period, which resulted in a very high NDVI and CVI, lower AI, and a balanced leaf nitrogen level (Figures 2.2. and 2.3.). In contrast, Tree 76226000 received full irrigation during the early season, and underwatering during the dry period did not show high foliage health based on the results of the aforementioned indexes (Figures 2.2. and 2.3.).

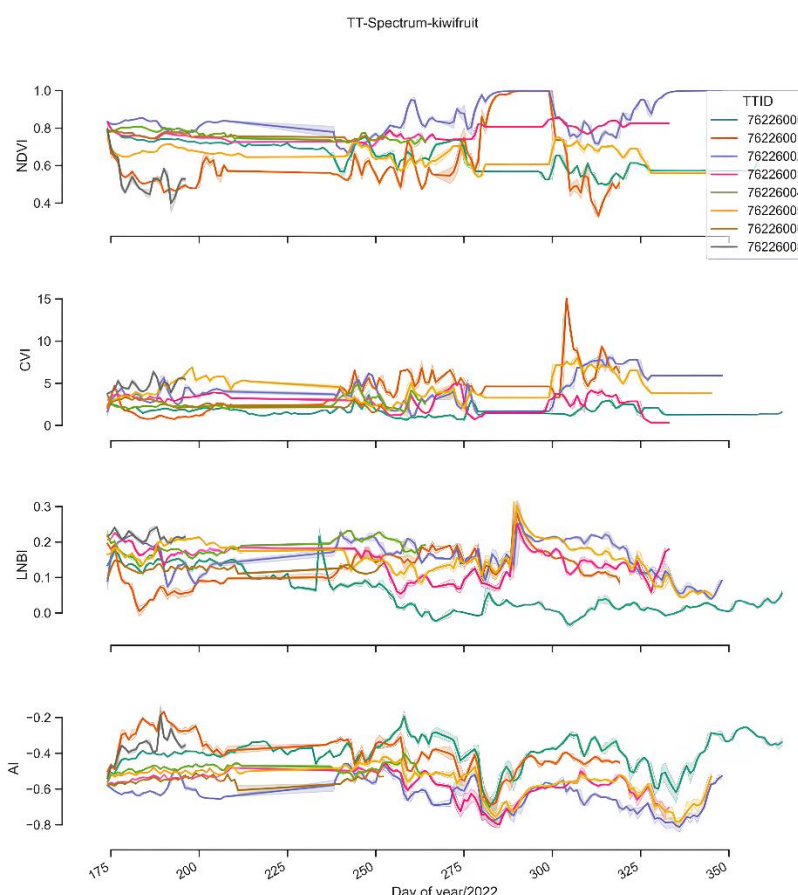


Figure 2.3. TT-Spectrum output showing the main vegetation indices — NDVI (Normalized Difference Vegetation Index), CVI (Chlorophyll Vegetation Index), LNBI (Leaf Nitrogen Balance Index), and AI (Anthocyanin Index) — for each individual, based on reflectance data from various spectral bands. These indices provide insights into plant health, chlorophyll content, nitrogen balance, and anthocyanin levels, respectively

2.3.2. *Effects of water deficit on ‘Soreli’ kiwifruit Chemical-Physical Parameters and Bioactive Compounds*

SSC of stored fruits showed a notable increase over time across all treatments (Table 2.1.), while the percentage of WL increased over time. No significant differences were observed between the various irrigation treatments, as previously demonstrated by Grasso et al. (2022). However, fruits subjected to full irrigation (100% water) exhibited lower sugar levels compared to those under deficit

irrigation. There were no significant differences in Brix° values for the samples with 60% and 80% water irrigation. The firmness, measured by both destructive and non-destructive methods, progressively decreased for all harvested fruits during cold storage under all irrigation conditions. Throughout the storage period, all samples consistently showed a softening tendency, indicating a uniform trend overall. Concerning the color parameters, L*, a*, and b* values at harvest (0 d) were highest in fruits irrigated with 100% water compared to fruits irrigated with 80% and 60% water. However, the results revealed a decreasing and homogeneous trend until the end of the study, with no significant differences between the different irrigation levels. The chromaticity coordinate a* consistently exhibited negative values that remained nearly constant throughout the storage time for all harvested fruits. Titratable acidity values did not differ among the different fruit samples during cold storage (Table 2.1).

Table 2.1. Physio-chemical parameters in fruits harvested analysed at 0 days, 15 days, 30 days, 45 days, 60 days and 75 days cold storage at 1 °C. Three technical replicates were realized for each biological sample

Parameter	Treatments	T0 (0 d)	T1 (15 d)	T2 (30 d)	T3 (45 d)	T4 (60 d)	T5 (75 d)
SSC (°Brix)	100%	8.72±0.55 (iA)	11.32±0.90 (ghA)	12.21±1.04 (fgB)	12.64±0.65 (efB)	14.18±0.37 (bcB)	14.34±0.91 (abB)
	80%	9.16±0.50 (iA)	11.44±0.62 (ghA)	13.30±0.61 (cdeA)	13.47±0.38 (cdA)	14.95±0.49 (abA)	14.80±0.66 (abA)
	60%	9.15±0.60 (iA)	11.08±0.91 (hA)	13.18±0.51 (defA)	13.65±0.41 (cdA)	15.10±0.50 (abA)	15.31±0.40 (aA)
FFP (kg cm ⁻²)	100%	7.61±1.81 (aA)	5.78±1.53 (bcA)	4.96±1.36 (cdA)	3.51±1.14 (deA)	1.97±0.84 (fghA)	0.71±0.48 (hA)
	80%	7.65±2.24 (abA)	5.75±2.20 (bcA)	4.36±2.07 (cdeA)	3.41±1.81 (efgA)	1.80±0.83 (hA)	0.95±0.48 (hA)
	60%	6.83±2.33 (aA)	5.80±1.69 (bcA)	4.40±1.54 (cdeA)	3.27±1.34 (efA)	1.60±0.53 (ghA)	0.81±0.42 (hA)
TA (mg citric acid/100g FW)	100%	4.00±0.87 (abA)	4.6±0.89 (aA)	3.30±1.04 (bB)	4.15±1.02 (abA)	4.10±0.94 (abA)	4.45±0.81 (abA)
	80%	3.85±0.75 (abA)	3.40±1.06 (bB)	4.30±0.95 (aA)	4.55±0.89 (aA)	3.45±1.08 (bB)	4.45±0.72 (aA)
	60%	4.35±0.68 (abA)	3.9±0.76 (bAB)	4±0.72 (abA)	4.4±0.84 (abA)	3.75±0.91 (bAB)	5.4±1.02 (aA)

Capital letters indicate comparisons between the maturity stages of fruits subjected to different treatments at each specific time. Small letters reflect comparisons between different storage times for each sample fruit of the same treatment. According to the Tukey test ($P < 0.05$), there are no significant differences between means that share the same letter within the same column or row

Analyzing the content of bioactive compounds, such as ascorbic acid (AA), carotenoids (CAR), chlorophyll a (Cl a), chlorophyll b (Cl b), flavonoids (FLAV), and polyphenols (POL), in fruits harvested and analysed at 0 days, 15 days, 30 days,

45 days, 60 days, and 75 days of cold storage at 1 °C, the data provide evidence that a reduction in water does not negatively influence the bioactive compounds of yellow fruits. This observation aligns with another experiment conducted by Nora et al. (2012). However, the change in content remains consistent across all irrigation methods. The changes in bioactive compound during the ripening of kiwifruits do not appear to differ significantly between the various irrigation techniques.

2.4. Conclusion

In conclusion, this study highlights the significant impact of soil moisture availability on the ecophysiological responses of kiwifruit plants. The study demonstrates that excessive irrigation may not always be beneficial for plant performance, as evidenced by the combination of vegetation spectral indices and fruit quality. In fact, by utilizing soil sensors and a spectrometer, we were able to correlate soil moisture data with vegetation indices, revealing the influence of varying water availability on plant health, growth, and productivity. It was observed that the CVI and LNBI values for leaves were higher in deficit irrigation, particularly at 80% water availability, compared to full irrigation. For the fruit, the soluble solids content was higher in deficit irrigation than in 100% irrigation, enhancing the desirable quality of the fruit that is appreciated by consumers and is further accentuated when irrigation water is reduced. In terms of all the analysed characteristics, including bioactive components and chemical and physical parameters of the fruit, no significant differences were found between the different irrigation conditions. This suggests that by reducing irrigation water, companies can achieve economic savings and reduce water waste while still obtaining high-quality final products. Based on the conducted study, it has been determined that employing 80% deficit irrigation yields superior results in terms of fruit quality and overall plant health. This could be a significant economic advantage for fruit and vegetable companies, as they can utilize IoT sensors to monitor the plant's condition and intervene as necessary. These insights can help inform targeted irrigation management practices, optimize water use, and promote sustainable growth in kiwifruit ecosystems. The study also emphasizes the importance of maintaining adequate soil moisture levels, especially during dry periods, to ensure optimal nutrient uptake, root development, and overall plant health.

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CHAPTER III

This chapter, closely linked to the previous chapter, describes the analyses carried out in the second year of my PhD research project (2023-2024). This study, currently being written, presents the results from the second growing season which builds upon the findings from the first-year experimentation (Chapter II). In this phase, we repeated and confirmed the data previously obtained on *Actinidia chinensis* 'Soreli' kiwifruit plants under different irrigation treatments. Additionally, the study was expanded by integrating new parameters, including sap flow measurements and the analysis of vapor pressure deficit (VPD), to gain a deeper understanding of plant physiological responses. The collected data and the resulting insights are discussed in detail in this chapter.

3. Application of IoT-TT for Monitoring Sap Flow in ‘Soreli’ Kiwifruit Plants and Assessing Fruit Quality

3.1. Introduction

Water scarcity is a growing global issue driven by rising consumption, inadequate management, and pollution. Agriculture plays a significant role in exacerbating this problem, primarily due to inefficient irrigation practices. This inefficiency is measured as the ratio between the water absorbed by crops and the total volume withdrawn for irrigation. Consequently, enhancing the efficiency of water use in crop irrigation has become a pressing focus for researchers and agronomists in recent years (Costa et al., 2007). Deficit irrigation strategies have the potential to optimize water productivity in horticulture, for example drip irrigation, mulching and protected cultivation have significantly reduced runoff and evapotranspiration losses (Jones, 2004; Kirnak & Demirtas, 2006; Stanghellini et al., 2003). Nevertheless, the effects of deficit irrigation on yield or harvest quality are crop-specific (Costa et al., 2007). Deficit irrigation strategies have been successfully used on fruit trees (Asgharinia et al., 2023; dos Santos et al., 2007; Lembo et al., 2023; Yang et al., 2011). The sensitivity of kiwifruit to soil water availability has been well documented and concerns different characteristics of the fruit. It is known that a significant reduction in harvest weight occurs if the supply of irrigation water is limited over summer. This effect is particularly severe if vines are drought stressed early in the growing season when fruit are in their most rapid phase of expansion. Otherwise, a mild drought stress can result in beneficial effects on the post-harvest storage characteristics of the fruit; when vines were given a mild drought stress early in the growing season, the fruits were firmer and had a better post-harvest

conservation than fruit from full irrigated vines (Miller et al., 1998). Furthermore, an adequate reduction of irrigation water has also proven positive on the development of sugar content. This accumulation is a recognized physiological response to water stress and has been demonstrated for several crops including peach (Steinberg et al., 1989), cherry (Ranney, 1991), strawberry (Zhang & D. D. Archbold, 1993) apple (Wang & Stutte, 1992), in kiwifruits (Lembo et al., 2023) and in tomato fruits (Ripoll et al., 2014). Larger irrigation volumes have been also proven to decrease melon fruit quality, especially total soluble solid content (Sensoy et al., 2007). It is possible that other components of fruit besides carbohydrates are affected by the stress treatments. Walton and De Jong (1990) found that stressed kiwifruit had higher concentrations of organic acids, suggested that sugar acid ratios may be a useful addition to current maturity testing standards (Walton & De Jong, 1990).

Moreover, severe water stress can cause serious problems for plants, contributing to the most serious phenomenon of mortality. As regards the health of the kiwi plant following controlled water stress, a reduced rate of photosynthesis caused by reduced stomatal conductance was observed. Furthermore, severe water stress appears to cause a reduction in plant height, total dry weight, and total leaf area. The decrease in growth induced by water deficits is a consequence of a reduction in both photosynthesis and photosynthates partitioning, which adversely affects leaf area development. It seems though that water stress in kiwifruit alters the pattern of dry matter distribution favouring the roots (Chartzoulakis et al., 1993; Gucci et al., 1996).

Plant water stress can be measured indirectly in several ways (e.g., leaf or stem water potential, tissue water content, variation of stem or fruit diameter); however, it is difficult to measure any of these parameters automatically over the long term. One indicator of plant water stress that can be measured automatically over the long term is sap flow. Sap flow density flux describes water transport in trees and most methods that have been used to measure sap flow were developed to provide accurate estimates of total tree water supply. The large amount of water supplied for transpiration, and thus evaporative cooling of leaves during the day, corresponds to the actual evaporative demand. Such water comes mostly from soil, but also partly from internal water storage. The internal store of water that is partially utilized during the day is replenished by sap flow during the night. Nighttime sap

flow is low compared to daytime sap flow (Asgharinia et al., 2022; Nadezhdina, 1999).

In the last decade, monitoring systems, which can be treated as a part of smart technologies and are used for generating large amounts of data sets from a network of sensors, have evolved rapidly. TreeTalker (TT+) is a device that collects information about the health state of the trees based on various internal and external factors and it is used for real-time monitoring made with the concept of the Internet of Things (IoT). It is responsible for collecting data from all sensors, which are fixed to trees, and transforming the analog signal to meaningful variables. It includes a large amount of information on the sap density flux describing water transport process in different tree species that also differ in age, health status, metric characteristics, etc (Efrosinin et al., 2021). Thermal approaches are an important category for determining sap flow density (SFD). The TT+ sap flow measurement technique is based on the temperature difference between a pair of probes. One of the probes is heated, while the other is used to measure the stem temperature and stem water content. TT+ sap flow measurement is designed based on the transient thermal dissipation technique with a cyclic heating system (Asgharinia et al., 2022). The main goal of the study was to evaluate the influence of irrigation patterns (100%, 80% and 60% of the conventional use) in determining water consumption and potential water stresses on *Actinidia chinensis* ‘Soreli’ Kiwi plants which could affect the quality of their kiwifruit production. Specifically, our research focuses on evaluating the effects of different irrigation levels on sap flow, a key physiological indicator reflecting the plant’s water transport efficiency and overall hydration status (Asgharinia et al., 2020).

By analyzing sap flow, we aim to understand how water availability influences internal water dynamics, which can directly affect plant health and resilience.

Additionally, we investigate how these irrigation variations impact post-harvest fruit quality, examining attributes such as fruit size, sugar content and shelf-life. This study seeks to provide valuable insights into optimal irrigation practices that could enhance both plant vitality and fruit quality, contributing to improved water-use efficiency in kiwi cultivation and potentially informing sustainable agricultural practices.

3.2. Materials and Methods

3.2.1. Experimental Design

Three experimental plots of *Actinidia chinensis* ‘Soreli’ kiwifruit vineyards at the "Tre Colli" farm in Cisterna Campoleone, Velletri, Rome, Italy, were selected to evaluate the effects of water deficit on plant sap flow and fruit quality. The plots share identical soil mineral composition and agricultural management practices. They were subjected to three distinct irrigation treatments: standard drip irrigation at 50-100 L daily (based on seasonal and weather conditions), designated as 100% or full irrigation; moderate deficit irrigation at 80% of the full irrigation level; and higher deficit irrigation at 60%. Each plot spans roughly 1 hectare, housing around 700 vines per hectare, with a spacing arrangement of 4.0 meters between rows and 3.0 meters between individual plants within rows.

3.2.2. The TreeTalker Platform

To evaluate the impact of varying irrigation volumes on plant physiology, low-cost IoT devices called TreeTalkers (TT+) were deployed. These devices, provided by Nature 4.0 S.r.l. (Viterbo, VT, Italy), measure physiological parameters such as sap flow by measuring movement of water within plants, typically in tree stems. These techniques are essential in fields like forestry, agriculture, and environmental science to understand plant water use, transpiration rates, and how plants respond to environmental stresses (e.g., drought, heat).

We have used the heat pulse velocity (HPV) method. This method measures the velocity of a heat pulse as it moves through the tree stem. A short heat pulse is generated by a probe inserted into the stem, and downstream sensors detect the pulse’s movement. The speed of heat movement through the stem is then used to estimate sap flow rate. It is suitable for measuring low and high flow rates and offers accurate point measurements. Sap flow techniques are crucial for understanding how plants interact with their environment, especially in the context of climate change and water resource management.

Among the available thermal methods to measure sap flux density, we are using a novel cyclic heating system with a new flow index developed by Do and Rocheteau (2002).

Cyclic heating system works in transient thermal dissipation (TTD) conditions by introducing a relatively short cycle of heating and cooling (a minimum of 10 minutes heating and 10 minutes cooling) (Do et al., 2011). The transient signal (dT) is the relative change in temperature over the heating period, between the differential cooled temperature (ΔT_c , after cooling) and the maximum differential temperature reached after the period of heating (ΔT_h) (Asgharinia et al., 2022):

$$dT = \Delta T_h - \Delta T_c$$

Where:

$$K_1 = \frac{dT_{\max} - dT_i}{dT_i}$$

And the sap flux density ($l\ dm^{-2}\ hr^{-1}$) is then calculated as:

$$SFD = 12.95 * K_1$$

3.2.3. Kiwi fruit storage conditions

In September, ‘Soreli’ kiwifruits were picked at 9 ± 2 °Brix value, ensuring they were uniform in size and appearance and free from defects and disease. After harvesting, the fruits were packed in single-layer trays and swiftly transported to the Postharvest Laboratory of DIBAF at the University of Tuscia in Viterbo, Italy. Initially, the kiwifruits were kept at room temperature for 24 hours before being cooled to $1 \pm 0.5^\circ C$ with a relative humidity of $85 \pm 5\%$ in a normal atmosphere, utilizing an ethylene absorber. Analyses were conducted on frozen fruit for six sampling times, at the time of harvest and every 15 days during cold storage. Analyses were conducted on fruit for seven sampling times, at the time of harvest (T_0) and every 15 days of sampling time during cold storage. (T_1 , T_2 , T_3 , T_4 , T_5 and T_6).

3.2.4. Physical-Chemical Parameters

At harvest (T_0) and during storage (T_1 - T_6), twenty kiwifruits were selected and weighed using a digital balance (Adam Equipment Co., Ltd., Milton Keynes, UK) to monitor weight loss (WL) during cold storage at $1 \pm 0.5^\circ C$. The same twenty fruits were weighed at each sampling time and were numbered beforehand to ensure the analysis was performed on the same fruits throughout the entire duration of the test.

The total soluble solid content (SCC) of the fresh kiwifruit juice was measured using a digital refractometer (ATAGO, Palette PR-32, Tokyo, Japan) and expressed as °Brix (%). Flesh color was evaluated on peeled fruits using a Minolta colorimeter (Minolta C2500; Konica Minolta, Ramsey, NY, USA) to assess the chromaticity values L* (Lightness), a* (green to red), and b* (blue to yellow). Chroma (C*) values were calculated as reported by McGuire (1992). The titratable acidity (TA) was measured in triplicate on the flesh juice obtained from five fruits following the protocol of Grasso et al. (2022).

3.2.5. *Bioactive Compounds Content*

Bioactive compounds were extracted from kiwifruit flesh in methanol solution (80% v/v) and the extract was centrifuged (14 000×g for 5 min). Total phenolic (TP) and flavonoid (TF) content was determined with Lambda 25 UV–Vis spectrophotometer (Perkin Elmer Instruments Ltd., Seer Green, Beaconsfield, U.K), according to the Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively. The TP content was expressed as milligrams of gallic acid equivalent (GAE) per 100 g–1 fresh weight (FW) and TF as milligrams of catechin equivalent (CE) per 100 g–1 fresh weight (FW) (Goffi et al., 2019). Chlorophyll content were determined following the procedure described by Lembo et al. (2023). Pigments were extracted using acetone-hexane solution (4:6 v/v), and the absorbance of the supernatant was measured using a spectrophotometer (Perkin Elmer Instruments Ltd., Seer Green, Beaconsfield, U.K). The results expressed as milligrams per 100 g fresh weight (FW).

3.2.6. *Statistical Analyses*

All data are presented as mean values $\pm$ standard deviation (SD). Statistical differences across fruit ripening stages, orchard locations, and storage durations were assessed using a two-way analysis of variance (ANOVA). Tukey's test was applied at a 5% significance level to evaluate differences between means. Results were considered statistically significant at $P < 0.05$, with distinct letters indicating significant differences.

3.3. Results and Discussion

3.3.1. Sap flow analysis of irrigated Kiwi fields

The results of the monitoring of sap flow rates were obtained in two consecutive seasons (2022 and 2023). In the year 2022 the differences in transpiration rates among the irrigation treatments were not so much different between the 100% and 80% rate while the 60% rate indicated a slight decline of transpiration on the basis of the average water consumption between the month of June to end of August (Figure 3.1.).

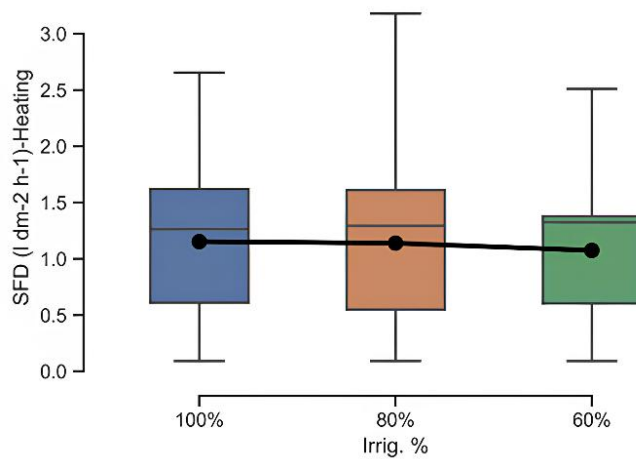


Figure 3.1. The box plot graph indicates the average sap flow density in $l\ dm^{-2}\ h^{-1}$ of water in different irrigation treatments (100%, 80% and 60% of the conventional use) in the year 2022

The analysis of the relation between the transpiration rate and the Vapour Pressure Deficit (VPD) which is the measure of the evaporative demand of the atmosphere, shows that the 100% irrigated trees at the same evaporating demand (VPD) show higher transpiration, while the other 2 treatments (80 and 60%) show reduced rates of transpiration (Figure 3.2.). This is explained by a moderate stomatal closure of these two irrigation regime compared to the highest irrigation treatment. However the stomata response occurs only at quite high VPD conditions ($>3\ KPa$) which are less frequent along the season. This also explain that the overall average transpiration rates are similar in the 100 and 80% regimes and only the 60% show a clear reduction behavior.

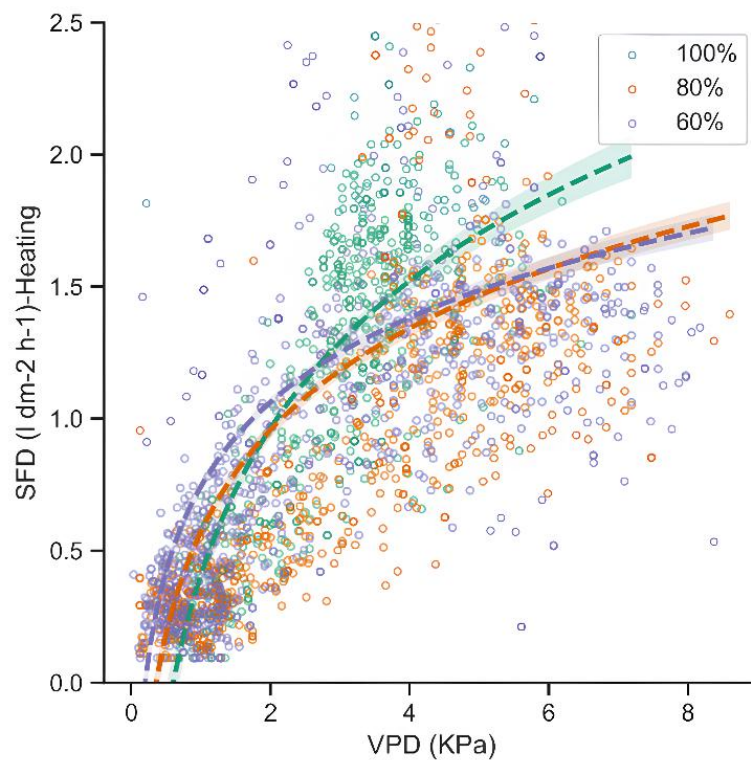


Figure 3.2. Relationship between the evaporative demand expressed by vapor pressure deficit (VPD) and sap velocity of kiwi trees in the year 2022

The same analysis was repeated in the year 2023 (Figure 3.3.) and results confirmed the 2022 general trend. The 100% and 80% treatments are similar in terms of water transpiration while a small decline occurs only in the 60% irrigation regime.

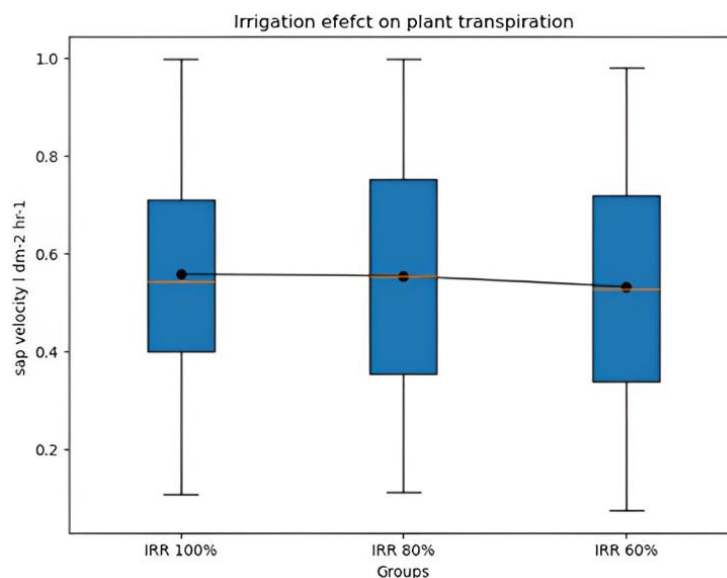


Figure 3.3. The box plot graph indicates the average sap flow density in l dm⁻² h⁻¹ of water in different irrigation treatments (100%, 80% and 60% of the conventional use) in the year 2023

3.3.2. *'Soreli' kiwifruit Chemical-Physical Parameters and Bioactive Compounds*

The study highlights the beneficial effects of deficit irrigation, specifically at 60% and 80% of full irrigation, on various quality parameters in yellow kiwifruit during storage. Notably, WL measured over the 2022-2023 seasons showed no significant difference across irrigation treatments (Figure 3.4.), aligning with findings by Grasso et al. (2022). Despite reduced sap flow and transpiration rates in water-stressed plants, the ultimate hydration levels in harvested fruits remained comparable, indicating that lower irrigation does not adversely impact WL during storage. Since all fruits experienced approximately 5% WL by the end of the storage period, it appears that preharvest irrigation regimes do not substantially affect WL outcomes during storage as already shown by other studies (Conesa et al., 2021; Goffi et al., 2019; Lembo et al., 2023).

Acid content, measured as titratable acidity (TA), also remained stable across irrigation treatments. This stability, observed consistently in both the 2022 and 2023 seasons, suggests that water availability has minimal influence on the fruit's acid composition during storage, in agreement with studies by Conesa et al. (2021), Goffi et al. (2019), and Lembo et al. (2023).

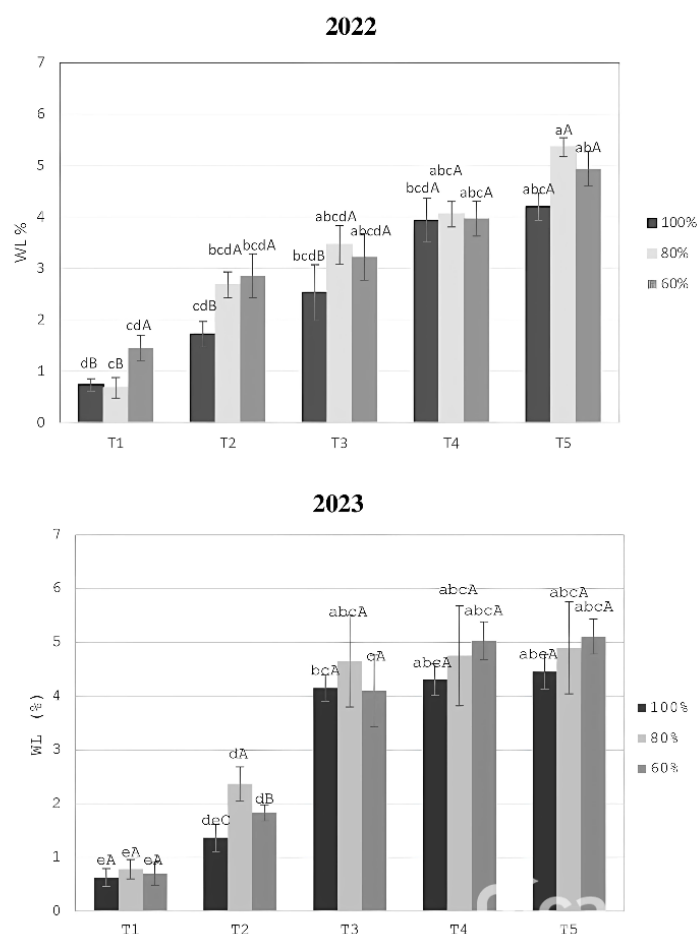


Figure 3.4. Weight loss (WL%) of ‘Soreli’ kiwifruit under three irrigation volumes (100%, 80%, and 60%) at harvest (T0) and at all sampling times in cold storage at 1 ± 0.5 °C during the 2022 and 2023 growing seasons. Data are presented as histograms for each year, highlighting the differences in post-harvest weight loss across irrigation treatments of cold storage at 1 ± 0.5 °C. Means depicted with the same capital letters do not show significant differences between different treatments at the same sampling time. Means depicted with the same lowercase letter are not significantly different from each other for the specific treatment. Error bars indicate standard deviations and values with different letters are significantly different ($P < 0.05$; Tukey test)

Soluble solids content (SSC), however, exhibited a marked increase across all treatments during storage, with fruits under deficit irrigation showing higher sugar levels compared to fully irrigated ones (Table 3.1.). This trend, where moderate water stress promotes sugar accumulation, is consistent with prior findings in kiwifruit (Zheng et al., 2023), as well as in other fruits like plum (Maatallah et al., 2015), pomegranate (Volschenk, 2021) and apple (Reid & Kalcsits, 2020).

Table 3.1. SSC in fruits harvested analysed at 0 days, 15 days, 30 days, 45 days, 60 days and 75 days cold storage at 1 °C in 2022 and 2023. Three technical replicates were realized for each biological sample

Parameter	Treatments	T0 (0 d)	T1 (15 d)	T2 (30 d)	T3 (45 d)	T4 (60 d)	T5 (75 d)
SSC 2022	100%	8.72±0.55	11.32±0.90	12.21±1.04	12.64±0.65	14.18±0.37	14.34±0.91
		(iA)	(ghA)	(fgB)	(efB)	(bcB)	(abB)
	80%	9.16±0.50	11.44±0.62	13.30±0.61	13.47±0.38	14.95±0.49	14.80±0.66
		(iA)	(ghA)	(cdeA)	(cdA)	(abA)	(abA)
	60%	9.15±0.60	11.08±0.91	13.18±0.51	13.65±0.41	15.10±0.50	15.31±0.40
		(iA)	(hA)	(defA)	(cdA)	(abA)	(aA)
SSC 2023	100%	11.30 ± 0.74	12.56 ± 1.02	13.85 ± 0.79	13.84 ± 0.72	14.39 ± 0.46	14.32 ± 0.95
		(ghA)	(efgA)	(bcdA)	(bcdA)	(abcB)	(abcB)
	80%	11.91 ± 1.10	13.02 ± 0.66	12.97 ± 0.54	14.29 ± 0.57	15.65 ± 1.15	14.47 ± 1.28
		(fgA)	(cdefA)	(cdefAB)	(abcA)	(aA)	(abcB)
	60%	10.20 ± 0.41	11.80 ± 0.64	12.68 ± 0.44	13.75 ± 0.58	13.59 ± 0.75	13.52 ± 1.34
		(hB)	(fgB)	(defgB)	(bcdeA)	(bcdB)	(aA)

Note: Capital letters indicate comparisons between the maturity stages of fruits subjected to different treatments at each specific time. Small letters reflect comparisons between different storage times for each sample fruit of the same treatment. According to the Tukey test ($P < 0.05$), there are no significant differences between means that share the same letter within the same column or row

Regarding color attributes, the study observed a general decrease in flesh brightness (L^*) across all treatments during storage, with final L^* values lower in fruits under reduced irrigation. This reduction suggests that water stress may accelerate the natural darkening that occurs during kiwifruit storage (Miller et al., 1998). The a^* values, indicating green-to-red intensity, became less negative over time, reflecting typical chlorophyll loss during ripening. The 60% irrigation group maintained more negative a^* values, indicating a slower loss of green pigmentation compared to higher irrigation levels, possibly due to stress-induced chlorophyll retention. The study also observed that b^* values, which represent the yellow component of color, were higher in fruits from the 100% irrigation treatment at both the beginning and the end of storage compared to those in the 80% and 60% irrigation treatments. This trend suggests that full irrigation promotes a more intense and desirable yellow coloration in kiwifruit. In summary, these findings indicate that water-deficit conditions induce shifts in pulp color characteristics, while higher irrigation levels are associated with a more favorable chromatic profile, featuring more intense yellow hues and greater brightness.

Additionally, the chroma values in the 100% and 80% irrigation treatments tend to be similar across most time points (T0–T6), while the values for the 60% treatment show a tendency to be higher at various points (such as T1, T3, and T6). This suggests that reduced irrigation can influence the color intensity of the fruit, resulting in higher chroma values (more intense color). In the reduced irrigation treatments, controlled water stress may induce stomatal closure and reduce transpiration, which could stimulate pigment synthesis, leading to a higher chroma value (more intense or saturated color) in the CIE Lab* system.

For chlorophyll content, fruits from the 60% irrigation treatment initially exhibited lower chlorophyll levels but maintained these levels consistently during the final stages of sampling (T5–T6), indicating an adaptive response to water stress. This trend aligns with findings by Pratima (2018), where water-stressed cultivars displayed a significant increase in chlorophyll stability index (CSI), demonstrating enhanced resistance to chlorophyll degradation under deficit irrigation. Early increases in chlorophyll are typical in yellow kiwifruit as they ripen, but the subsequent decrease reflects the expected degradation and metabolic shifts during ripening (Botondi et al., 2021; Goffi et al., 2019).

Moreover, the polyphenol and flavonoid content in fruit showed an increase under water-deficit conditions. At harvest, polyphenol levels peaked in fruits from the 60% irrigation group, reaching over 200 mg GAE/100 g FW at T2. Flavonoid levels also showed a significant increase in the 60% irrigation treatment, particularly at T2, suggesting that moderate water stress enhances the synthesis of these antioxidant compounds.

3.4. Conclusion

This study underscores the positive impact of deficit irrigation on enhancing the quality of yellow kiwifruit and on the health of the plant, in particular with regard to sap flow and transpiration rate, supporting it as a viable alternative to full irrigation.

We can conclude that the 100% irrigation regime seems to be an higher rate of water supply, more than the plant really needed. This is shown by the fact that the 80% irrigation scheme maintain in both years the same transpiration rate. Only the 60% seems to have an effect. Furthermore, the findings from this study underscore that reduced irrigation triggers physiological adjustments, such as moderate stomatal closure and altered metabolic pathways, that contribute to improved fruit characteristics without adversely affecting key attributes like WL and TA during storage. The 60% irrigation treatment showed a capacity to promote SSC accumulation and intensify desirable chromatic qualities, particularly yellow pigmentation, which aligns with consumer preferences for vibrant color. The observed stability in chlorophyll content under water stress also suggests a delayed degradation process, likely contributing to the retention of greener tones and enhanced visual appeal. Furthermore, increased levels of polyphenols and flavonoids in fruit under water stress emphasize the potential for improved nutritional value through regulated water deficit, enhancing antioxidant content. These findings highlight deficit irrigation as a strategy that not only conserves water resources but also enhances key quality parameters, making it both economically and environmentally advantageous for kiwifruit production. Since the quality of kiwifruits is not affected in 100% and 80% irrigation rates, this study provide strong evidence that water can be saved up to 20% without affecting the production quality and quantity, supporting strongly economic saving and at same time sustainability of the Kiwi production in future climate changes. It may be however necessary to say that in some extreme events an emergency irrigation still should be considered.

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CHAPTER IV

This article is currently being written and presents analyses conducted during the second and third years of my Ph.D. research. The study aims to evaluate the antioxidant activity of ‘Soreli’ kiwifruits subjected to different irrigation treatments, to assess whether water volume can influence enzymatic activity and antioxidant content.

4. Effect of the plant water deficit irrigation on the postharvest nutritional quality parameters and antioxidant enzyme activities of ‘Soreli’ kiwifruits

4.1. Introduction

Yellow-fleshed kiwifruit (*Actinidia chinensis*) is native to China and now grown in several countries of world including Italy, the first European kiwifruit producer with a production estimated at 523,000 tons, representing about 12% of global production (Delle et al., 2024).

Compared to green fleshed kiwifruit, the yellow-fleshed cultivars (cv) have popularity among consumers due to their sweeter taste, smooth skin, and rich nutritional profile (Zheng et al., 2023), including the higher vitamin C content, excellent equilibrium between sugar and acidity and a remarkable antioxidant pattern. (Goffi et al., 2021). Recent studies have demonstrated the potential health benefits of yellow-fleshed kiwifruit, including its role in enhancing immune function, supporting digestive health, and its possible anti-inflammatory properties (Richardson et al., 2018).

Among the yellow cv's, ‘Soreli’, released in 2008 by the University of Udine (Italy), is characterized by particular agronomic features such as high productivity, early ripening and large fruit size (Goffi et al., 2019).

Fruit ripening is a process that involves the regulation of various biochemical and physiological traits, as well as changes in oxidative metabolism. During storage, fruits produce reactive oxygen species (ROS) such as ($\text{OH}\cdot$), ($\cdot\text{HO}_2$), (O_2^-) and (O_3^-) ions, which can act as signals and secondary messengers for activating stress and defence pathways, but their accumulation can result in oxidative damage to biomolecules. The intercellular levels of ROS depend on the balance and between their generation and the ability to remove them due to antioxidant pathway that include both enzymatic and non-enzymatic systems. Non-enzymatic systems including ascorbic acid, polyphenols, carotenoids, anthocyanins and other

molecules work alongside enzymatic antioxidant systems characterized by key enzymes such as superoxide dismutase (SOD), peroxidases (in the ascorbate-APX, glutathione-GPX forms) and catalase (Botondi et al., 2021; Zhu et al., 2008).

It was observed that yellow-fleshed kiwifruit cv's are generally more perishable than green-fleshed ones and this could be explained by a lower activity of antioxidant enzymes, such as catalase (CAT), guaiacol peroxidase (GPX), superoxide dismutase (SOD), and ascorbate peroxidase (APX) in the yellow fruits (Goffi et al., 2020). However, the reduction of antioxidant content, the rapid softening and, in general, the rapid ripening time from harvest of 'Soreli' kiwifruits, makes it challenging to maintain quality and meet consumer expectations above all under inadequate postharvest storage or distribution conditions (Meitha et al., 2020). Addressing these concerns, applying several pre or postharvest strategies turns out to be essential for enhancing the competitiveness of this kiwifruit cultivar for the market (Goffi et al., 2019; Yang et al., 2020). Several studies evaluated controlled deficit irrigation to assess the effect on plant health, fruit quality during their storage (Lembo et al., 2023; Luis O. Lagos et al., 2017; Zheng et al., 2023) and to get a sustainable management system in horticulture fields, decreasing water waste, costs and environmental concerns (Ferreira et al., 2003). Limited water use efficiency is driving growers to implement advanced irrigation and cultivation strategies that optimize water utilization and improve crop yield. (Costa et al., 2007). Regulating irrigation can affect the quality of growth and the nutritional content of fruits and vegetables (Yang et al., 2017), increasing the content of soluble solids, titratable acids, vitamin C, while improving the firmness, shape and color of the fruit (Zhou et al., 2025). For example, Wang et al. (2021) evaluated in pear cv's how several low drip irrigation volumes had beneficial effects on some quality parameters, as on the good balance between soluble solids content and titratable acidity. Further studies also confirm in kiwifruit plants that water deficit irrigation can be useful to improve some fruit quality attributes (Judd & K. John McAneney, 1987; Miller et al., 1998). The reduction of irrigation water in horticulture can be provided with different approaches: both as the "classic deficit irrigation strategy" that implies that water is supplied at low levels throughout the season. and as "regulated deficit irrigation", which involves an intermittent water deficit which can be beneficial for the low water use efficiency, increase water savings and improve harvest fruit quality (Ferreira et al., 2003; Suárez et al., 2010).

Another approach is “partial root drying” which exposes roots to alternating water cycles, reducing water loss and vegetative growth while enhancing water use efficiency and influencing both fruit yield and quality. Grapevines and several fruit plants, as peach plant, appear to adapt well to deficit irrigation, whereas other crops, such as vegetables, often struggle due to reductions in yield and quality (Suárez et al., 2010). As a result, different irrigation management strategies can lead to notable variations in crop yield, fruit quality and the overall reduction in water usage.

The aim of this study is to investigate the impact of different volume contents delivered for plants irrigation on the postharvest fruit quality traits: in detail, the contents of bioactive compounds and the antioxidant enzymes activity of 'Soreli' kiwifruit during cold storage.

4.2. Materials and Methods

4.2.1. Experimental design

In the summer of 2023, three experimental plots of ‘Soreli’ kiwifruit vineyards at the "Tre Colli" farm in Cisterna Campoleone (Velletri, Rome, Italy) were selected to study the impact of water deficit on plant health and product fruit quality. This study replicated the analysis conducted in 2022 under the same conditions on the same plants (Lembo et al., 2023). Each plot shares the same mineral composition and cultivation practices but receives different irrigation treatments: standard drip irrigation at 50-100 L per day based on seasonal and climatic conditions (100% of full water irrigation) and, in the same sampling times; deficit at 80% and 60% of the full irrigation rate. Each plot covers about 1 hectare and contains approximately 700 vines, with plants spaced 4.0 m apart between rows and 3.0 m apart within rows.

4.2.2. Kiwi fruit storage conditions

‘Soreli’ kiwifruits were picked at 9 ± 2 °Brix value, ensuring they were uniform in size and appearance and free from defects and disease. After harvesting, the fruits were packed in single-layer trays and swiftly transported to the Postharvest Laboratory of DIBAF at the University of Tuscia (Viterbo, Italy). Initially, the kiwifruits were kept at room temperature ($20 \pm 2^\circ\text{C}$) for 24 hours (Figure 4.1.). Then, the fruits were cooled to $1 \pm 0.5^\circ\text{C}$ with a relative humidity of $85 \pm 5\%$ in a

normal atmosphere, utilizing an ethylene absorber. Analyses were conducted on fruit for seven sampling times, at the time of harvest (T0) and every 15 days of sampling time during cold storage. (T1, T2, T3, T4, T5 and T6).



Figure 4.1. ‘Soreli’ kiwifruits irrigated with three different water volumes of irrigation (100%, 80%, 60%), stored for 24 h at room temperature in single-layer boxes storage in cold storage

4.2.3. *Physico-chemical parameters*

Twenty kiwifruits were selected and weighed 24 hours after collection (T0) and during all subsequent samplings time (T1-T6) using a digital balance (Adam Equipment Co., Ltd., Milton Keynes, UK) to monitor weight loss (% of WL) during cold storage. Additional 20 samples were used for each of the determined destructive assays within the specified times of storage.

The soluble solid content (SCC) is expressed in °Brix (%) and is evaluated using digital refractometer (ATAGO, Palette PR-32, Tokyo, Japan). Flesh color was evaluated on half-open fruits (Figure 4.2.) using a Minolta colorimeter (Minolta C2500; Konica Minolta, Ramsey, NY, USA) to evaluate the following parameters: L* (Lightness), a* (green to red), b* (blue to yellow) and ΔE (the percentual difference between two colors). Fruit firmness was measured by removing a 1 mm thick slice of skin from the equatorial region of each kiwifruit and using a digital penetrometer (Model 53205; TR Turoni snc, Forli, Italy). The firmness values were recorded in Kg cm⁻². The titratable acidity (TA) was measured homogenizing 2 g of frozen sample tissue in distilled water and subsequently titrating each sample with an alkaline solution (NaOH, 0.1 N) until pH 8.2, following the protocol of Grasso et al. (2022).



Figure 41.2. Yellow kiwi fruits cut in half for CIELab destructive colorimetric analysis. The fruits were analysed according to the different irrigation volumes used (100%, 80%, 60% water)

4.2.4. *Bioactive compounds content and antioxidant activity*

Bioactive compounds content has been extracted following the method described by Goffi et al. 2019. The total polyphenol (POL) and flavonoid (FLAV) contents were determined using the Folin–Ciocalteu (Singleton and Rossi 1997) and the aluminum chloride colorimetric method (Zhishen et al 1999) with 20 and 200 μ l of extract, respectively. The results were expressed in mg gallic acid equivalent (GAE)/ 100 g fresh weight (FW) and mg catechin equivalent (CE)/100 g FW.

The ascorbic acid (AA) content in kiwifruit was measured using the method outlined by (Goffi et al., 2019). The results were expressed as mg of AA per 100 grams of fresh weight (FW).

Chlorophyll and β -Carotene contents were determined following the procedure described by Lembo et al. (2023). Pigments were extracted using acetone-hexane solution (4:6 v/v), and the absorbance of the supernatant was measured at 663 nm, 645 nm, 505 nm, and 453 nm using a spectrophotometer (Perkin Elmer Instruments Ltd., Seer Green, Beaconsfield, U.K). The results expressed as milligrams per 100 g fresh weight (FW).

The scavenging activity of the alcoholic extracts was evaluated using the 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay according to the method of Ferrara et al. (2024). The ABTS radical cation solution was prepared with an initial absorbance of 0.70 at 734 nm, then mixed with 5 μ l of kiwifruit extracts and the absorbance was recorded after 6 min incubation. Each sample was analysed in triplicate for technical accuracy.

Trolox (TE) at concentrations of 4, 8, 16 and 32 μM was used as a positive control. Results were expressed as μl TE per gram fresh weight (FW).

4.2.5. Enzyme extraction and activity assays

Total soluble proteins were extracted by re-suspending frozen fruit tissue powder in extraction buffer and 5 mM ascorbic acid (used only for ascorbate peroxidase (APX) extraction) as described by Goffi et al. (2019). The homogenate was then centrifuged at $18.000 \times g$ for 10 minutes at 4 °C, and the resulting supernatant was used to assess the activity of superoxide dismutase, catalase and ascorbate peroxidase. Protein content in each crude enzyme extract was determined by the Bradford assay (Bradford, 1976), using bovine serum albumin as the standard.

4.2.6. Catalase and ascorbate peroxidase activity

Catalase activity (EC 1.11.1.6) was measured using the reaction mixture described by Goffi et al. (2019) for ‘Soreli’ kiwifruit. The reaction was initiated with the addition of H_2O_2 , and the breakdown of H_2O_2 was tracked by monitoring the decrease in absorbance at 240 nm. Specific activity was calculated as the molar rate of H_2O_2 decomposition, expressed in $\mu\text{mol gFW}^{-1}$.

Ascorbate peroxidase activity (EC 1.11.1.11) was measured following the method by Goffi et al. (2019). The reaction was initiated by adding H_2O_2 , and ascorbic acid oxidation was monitored by observing the decrease in absorbance at 290 nm. Specific activity was expressed as the molar rate of ascorbate oxidation, in $\mu\text{mol g FW}^{-1}$.

4.2.7. Superoxide dismutase activity

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured based on its inhibition of the photochemical reduction of nitro blue tetrazolium (NBT) in the presence of riboflavin, as outlined by Goffi et al. 2019. The reaction was evaluated at 560 nm and one unit of SOD activity was defined as the amount of enzyme required to inhibit NBT reduction by 50% under these conditions, with specific activity expressed as U g FW^{-1} .

4.2.8. Hydrogen peroxide content determination

Hydrogen peroxide content was measured following the method described by Alexieva *et al.* (2001). The reaction mixture contained 0.1% trichloroacetic acid and 1 mol L⁻¹ potassium iodide and 300 µL of crude enzyme extract, and the absorbance of the mixture was recorded at 390 nm. Results were reported as nmol H₂O₂ per kg of fresh weight (FW).

4.2.9. Statistical analysis

For physical, chemical and enzymatic analyses, the results are given as mean values $\pm$ standard deviations. Statistical comparisons between samples were performed with two-way analysis of variance (ANOVA), followed by Tukey's test at a 5% significance. The mean values were considered significantly different when $P < 0.05$ and were marked with different letters.

4.3. Results and Discussion

4.3.1. Effect of different irrigations on physical and chemical parameters of 'Soreli' kiwifruit

A significant factor contributing to fruit kiwifruit deterioration during storage is the fruit water loss (Woods, 1990). In our experiment no significative differences in weight loss values between different irrigation conditions has been found (Table 4.1.). However, WL increase over time is observed for all samples and, at the end of the storage, WL reached approximately 5% lower of the initial value. These results are confirmed by further investigations carried out for WL of kiwifruits (Grasso *et al.*, 2022; Guldás, 2003; Lembo *et al.*, 2023) and other studies conducted on different types of fruit, such as apricot (Pérez-Pastor *et al.*, 2007) and pomegranate (Laribi *et al.*, 2013) in which it was observed comparable findings regarding the influence of irrigation on WL.

The measurement of flesh firmness using penetrometer shows a decrease during the storage period, as expected for ripening fruits (Chen *et al.*, 2024; Conesa *et al.*, 2021; Taglienti *et al.*, 2009). Initial values (T0) were the highest, with a firmness of approximately 8-9 kg cm⁻², whereas by T6, firmness declined to below 1 kg cm⁻², indicating a highly ripe and softened fruit (Figure 4.3.). During the early sampling times (T0-T2), no marked differences were observed between the different

irrigation conditions. However, at T3 and T4, kiwifruits irrigated with 60% water tended to maintain greater firmness compared to other treatments, as indicated by some studies on different fruit crops; in fact, it was observed that peach fruits from trees subjected to deficit irrigation were firmer than control fruits, but did not differ in weight and diameter (Alcobendas et al., 2013; Pérez-Pastor et al., 2007). This could suggest that kiwifruits subjected to water-deficit irrigation retain greater firmness during storage, possibly due to an increase in soluble solids content, reduced water loss, enhanced structural integrity at the cellular level and the accumulation of protective compounds. These adaptations strengthen the fruit's cellular structure, slowing softening processes and preserving firmness over time (Mpelasoka et al., 2001).

The SSC increased progressively for all treatments during storage, which indicates a natural process of ripening and accumulation of sugars in kiwifruits, as already shown by other studies (Gullo et al., 2016; Morra et al., 2022). During the storage time the °Brix values in fruits irrigated at 60% increased, reaching the highest levels at T6 (15.62 for 60%, compared to 14.72 for 100%) (Table 4.1). At T4, fruits irrigated with 80% water exhibited a significantly higher °Brix value compared to those irrigated at 100% and 60%. Reduced irrigation (60%) appears to lead to a slower but consistent increase in °Brix during storage. The works carried out by Alcobendas et al. (2013) and Navarro et al. (2010) on mandarin fruit confirmed our results. These findings imply that plants experiencing moderate water-deficit stress tend to accumulate higher levels of carbohydrates than those without water stress. Reduced water availability likely influences sugar composition by concentrating soluble solids due to lower water content (Navarro et al., 2010).

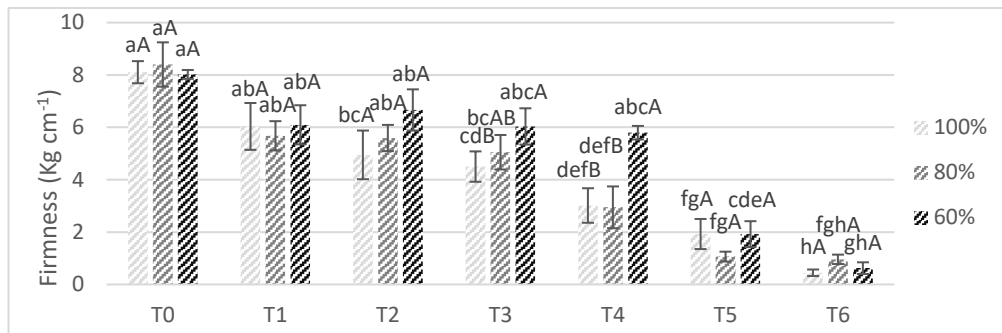


Figure 4.3. Firmness (Kg cm^{-2}) in ‘Soreli’ kiwifruits irrigated with 100%, 80% and 60% of water at harvest (T0) and at 15 days (T1), 30 days (T2), 45 days (T3), 60 days (T4), 75 days (T5) and 90 days (T6) of cold storage at 1 ± 0.5 °C. Means depicted with the same capital letters do not show significant differences between different treatments at the same sampling time. Means depicted with the same lowercase letter are not significantly different from each other for the specific treatment. Error bars indicate standard deviations and values with different letters are significantly different ($P < 0.05$; Tukey test)

TA values were similar in the three irrigation treatments as showed by Conesa et al. (2021) in nectarine fruit and did not exhibit significant changes during cold storage (data not show). Also Goffi et al. (2019) observed no significant changes on TA content of ‘Soreli’ kiwifruits during the cold storage.

The flesh color of yellow kiwifruit showed a decrease during the 90 days of storage at 1 °C for the CIELab coordinates L^* , b^* and a^* all of which parameters show a reduction from T0 to T6 (Figure 4.4.).

L^* values showed a similarity between the different treatments in the first sampling time, indicating that the fruits had a homogeneous brightness. At T6, L^* values continued to decrease across all irrigation volume treatments with 47.12, 43.98 and 43.17 for 100%, 80%, and 60%, respectively (Figure 4.4a). Also, in our previous study on ‘Soreli’ kiwifruits subjected to different irrigation conditions we observed a linear decreasing trend in the L^* parameter, indicating a decline in overall brightness (Lembo et al., 2023).

The a^* parameter in kiwifruits tends to lower negative values during storage, regardless of different irrigation volumes. This trend suggests a reduction in green intensity, potentially indicating a gradual loss of chlorophyll as the fruit ripe, which also happens in the leaves of plants (Matile et al., 1999). Kiwifruit subjected to 100% and 80% irrigation treatments exhibited a^* values less negative compared to those irrigated with 60% water.

Also, b^* values showed higher values for 100% irrigation treatment at the beginning and at the end of sampling (T0 and T6) when compared with 80% and 60% irrigation treatment, suggesting that adequate water availability promotes the development of desirable yellow coloration in the fruit across the initial and final storage times (Figure 4.4c).

These observations suggest that water deficit conditions induce changes in flesh color. Higher irrigation levels correlate with a shift toward a more favorable coloration profile, characterized by enhanced yellow hues (Figure 4.4b) (Alcobendas et al., 2013).

The CIELab color difference (ΔE) facilitates accurate assessment and enhances our understanding of the color changes in fruit that occur during cold storage time (Moschetti et al., 2019). For the ‘Soreli’ kiwifruit from plants irrigated at 100%, it was observed a greater variability in color difference, indicated by a high ΔE , particularly during the storage period at T3 (maximum value). In contrast, the fruits from the plants irrigated at 60% shows a more stable and reduced ΔE , suggesting a better color retain over time. The fruits from the plants subjected to 80% irrigation exhibit an enhanced variability compared to 100% treatment and 60% treatments (Figure 4.4d). Overall, the ΔE values indicate that the irrigation treatment significantly impacts on the color stability trend of ‘Soreli’ yellow kiwifruits, with the 60% fruit from 60% plant irrigation level yielding superior results over time.

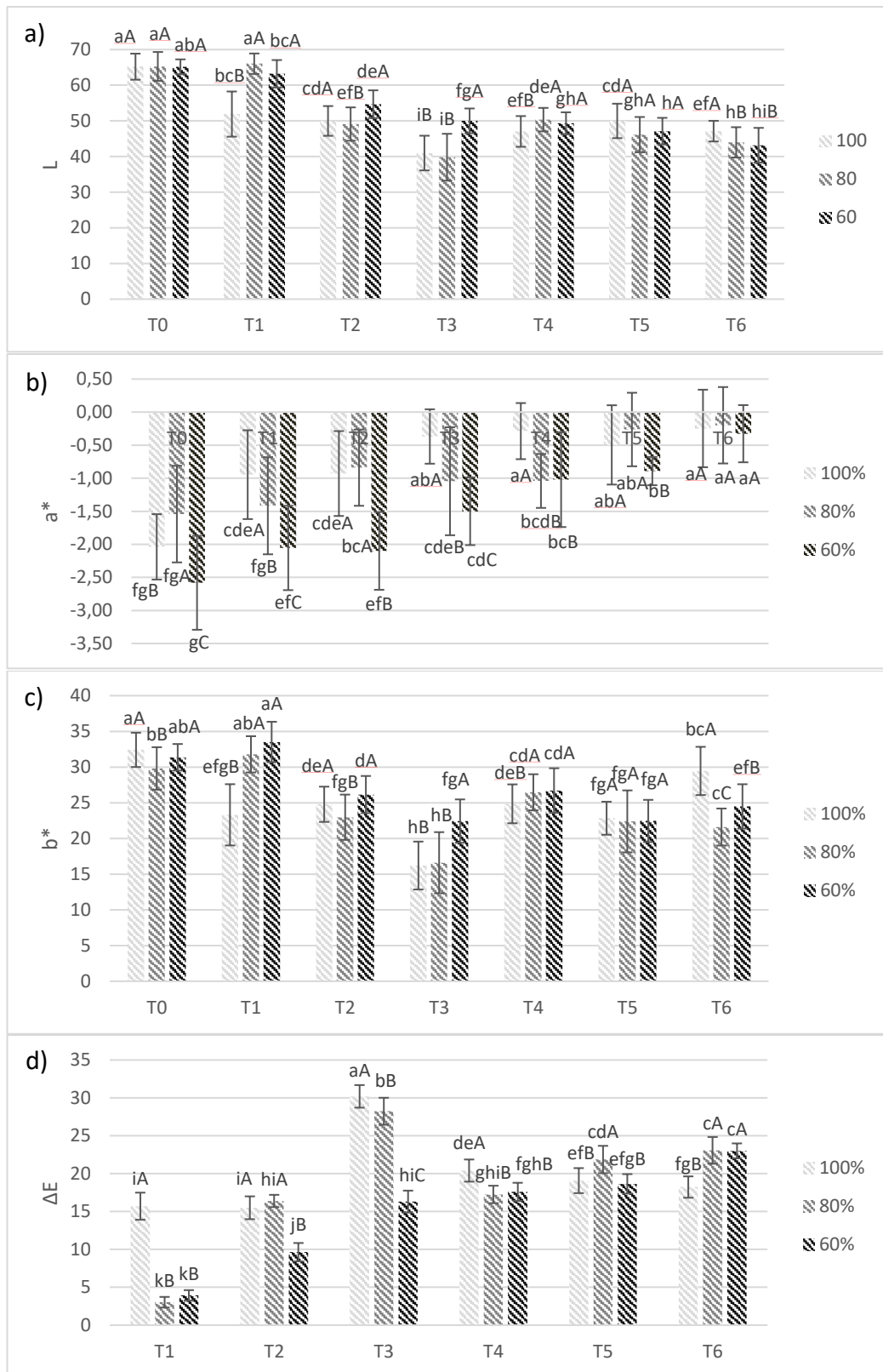


Figure 4.4. Color parameters (a) L*, lightness; (b) a*, Green-Red axis; (c) b*, Blue-Yellow axis; (d) ΔE: color difference in ‘Soreli’ kiwifruit irrigated with 100%, 80% and 60% of water at harvest (T0) and at 15 days (T1), 30 days (T2), 45 days (T3), 60 days (T4), 75 days (T5) and 90 days (T6) of cold storage at 1 ± 0.5 °C. Means sharing the same capital or lowercase letter are not significantly different. Different letters indicate significant differences ($P < 0.05$, Tukey test). Error bars show standard deviations

4.3.2. Effect of different irrigations on bioactive compounds of 'Soreli' kiwifruit

AA is renowned as an essential nutrient with unique health benefits in the human diet and different typology of fruit serves as a significant dietary source of this vital compound (Navarro et al., 2010). The literature indicates that kiwifruit AA content may reaches its maximum value in the full fruit ripening phase and subsequently stabilize or decrease during softening stages (Oz, 2010; Tavarini et al., 2008). This aligns with our results shown in Table 4.1. which demonstrate an increase in vitamin C content during the initial periods (from T0 to T3), followed by a subsequent stabilization. The fruits from plants irrigated with 60% of water reach their maximum AA content at T2, after which it stabilizes. In contrast, the fruits from plants irrigated with 100% water peak at T3 before stabilizing, while those from plants irrigated with 80% water exhibit their peak at T4. Nevertheless, it was possible to observe that the fruits from fully irrigated plants showed overall the highest AA values during the storage period. The fluctuation of antioxidants, specifically AA, can be affected by a number of different conditions as temperature, availability of sunlight, agronomic practices and irrigations techniques (Grasso et al., 2022). In contrast to our results, in the study conducted by Navarro et al. (2010) the AA content was higher in mandarin fruits harvested from plants subjected to water stress compared to control fruits and also in tomatoes it was observed that a reduction in water irrigation increased the fruit AA content compared to the full irrigated control (Martí et al., 2018). In addition to the high content of AA, kiwifruits are also rich in other antioxidants, such as phenolic compounds, flavonoids and carotenoids. These latter compounds are responsible for the yellow color of flesh and offer various health benefits. They can help scavenge reactive oxygen species, slow down the aging process, prevent cardiovascular and eye diseases, and strengthen the immune system in humans (Xia et al., 2020).

Table 4.1. Physio-chemical parameters in fruits harvested analysed at 0 days (T0), 15 days (T1), 30 days (T2), 45 days (T3) and 60 days (T4) cold storage at 1 °C. Three technical replicates were realized for each biological sample

Parameter	Treatment	T0	T1	T2	T3	T4	T5	T6
Weight Loss (WL %)	100%	-	0.63 ± 0.17 (eA)	1.37 ± 0.26 (deC)	4.15 ± 0.24 (bcA)	4.30 ± 0.29 (abcA)	4.45 ± 0.33 (abcA)	4.54 ± 0.43 (abcA)
	80%	-	0.78 ± 0.18 (eA)	2.37 ± 0.32 (dA)	4.65 ± 0.85 (abcA)	4.75 ± 0.93 (abcA)	4.90 ± 0.85 (abcA)	5.15 ± 0.87 (abA)
	60%	-	0.69 ± 0.21 (eA)	1.83 ± 0.15 (dB)	4.10 ± 0.67 (cA)	5.03 ± 0.35 (abcA)	5.11 ± 0.33 (abcA)	5.26 ± 0.29 (abA)
SSC (°Brix)	100%	11.30 ± 0.74 (ghA)	12.56 ± 1.02 (efgA)	13.85 ± 0.79 (bcdA)	13.84 ± 0.72 (bcdA)	14.39 ± 0.46 (abcB)	14.32 ± 0.95 (abcB)	14.72 ± 0.88 (abcAB)
	80%	11.91 ± 1.10 (fgA)	13.02 ± 0.66 (cdefA)	12.97 ± 0.54 (cdefAB)	14.29 ± 0.57 (abcA)	15.65 ± 1.15 (aA)	14.47 ± 1.28 (abcB)	14.46 ± 0.89 (abcB)
	60%	10.20 ± 0.41 (hB)	11.8 ± 0.64 (fgB)	12.68 ± 0.44 (defgB)	13.75 ± 0.58 (bcdeA)	13.59 ± 0.75 (bcdB)	13.52 ± 1.34 (aA)	15.62 ± 0.76 (aA)
AA	100%	31.44 ± 2.96 (gA)	32.61 ± 1.72 (fgB)	55.46 ± 0.91 (abcA)	62.26 ± 3.18 (abcA)	55.02 ± 0.35 (abcA)	55.56 ± 1.50 (aA)	56.11 ± 0.80 (aA)
	80%	36.82 ± 3.53 (efgA)	44.40 ± 2.81 (cdefA)	50.18 ± 0.76 (bcdB)	54.14 ± 0.76 (abcB)	56.41 ± 4.13 (abcB)	55.64 ± 1.90 (abcA)	50.89 ± 0.93 (abcB)
	60%	40.49 ± 2.30 (defgA)	40.92 ± 1.02 (defgA)	53.77 ± 1.94 (abcA)	51.25 ± 4.13 (abcdB)	47.51 ± 1.39 (cdeB)	46.30 ± 3.47 (cdeB)	46.38 ± 1.50 (cdeC)

Note: Capital letters indicate comparisons between different treatments of fruits at each specific time. Small letters reflect comparisons between different storage times for each sample fruit of the same treatment. According to the Tukey test ($P < 0.05$), there are no significant differences between means that share the same letter within the same column or row

As previously observed in the study by Grasso et al. (2022), on ‘Soreli’ kiwifruits, our experiment shows a clear decreasing trend in carotenoids content in the fruit flesh across all treatments during the storage period (Figure 4.5).

Fruits harvested from plant with 100% irrigation initially show the highest levels of β -carotene, suggesting that good water availability may promote early carotenoid synthesis. Fruits irrigated at 60% exhibit a smaller decline in carotenoids, indicating that moderate watering may help maintain carotenoid levels, though it may also limit synthesis.

In contrast, studies on cherry and goji berry varieties have shown that reduced irrigation can increase total carotenoid content, while results in tomato varieties

under water-deficit stress are consistent with our findings on ‘Soreli’ kiwifruit (Breniere et al., 2024; Coyago-Cruz et al., 2017).

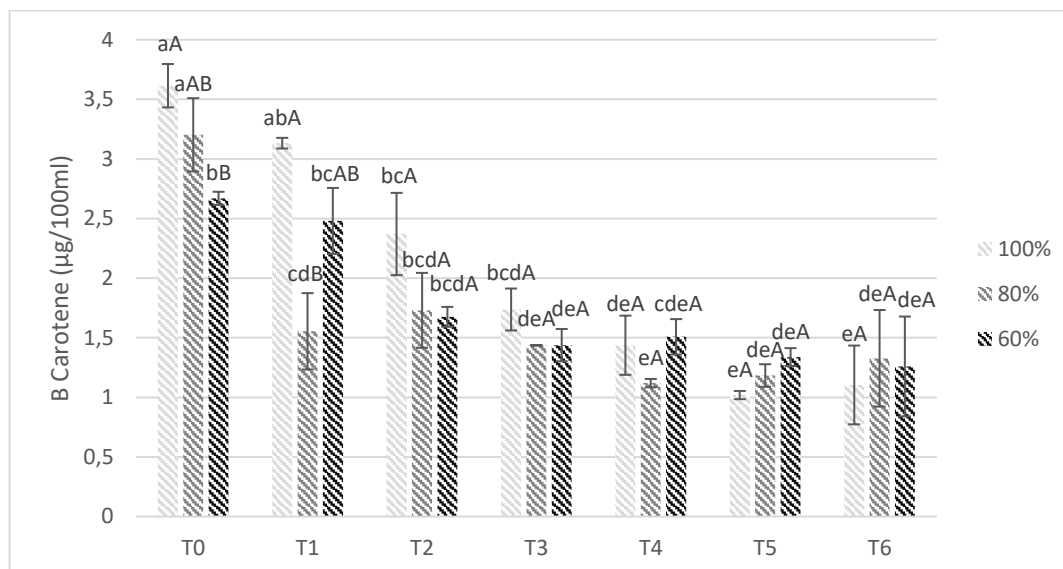


Figure 4.5. β -Carotene in yellow kiwifruit irrigated with 100%, 80% and 60% of water at harvest (T0) and at 15 days (T1), 30 days (T2), 45 days (T3), 60 days (T4), 75 days (T5) and 90 days (T6) of cold storage at 1 ± 0.5 °C. Means depicted with the same capital letters do not show significant differences between different treatments at the same sampling time. Means depicted with the same lowercase letter are not significantly different from each other for the specific treatment. Error bars indicate standard deviations and values with different letters are significantly different ($P < 0.05$; Tukey test)

Chlorophyll is found in various plant organs, including unripe fruits, and typically undergoes degradation during the ripening process (Pilkington et al., 2012). As can be seen in Figure 4.6. the Chl flesh content of ‘Soreli’ yellow fruit tends to increase during intermediate time (T2-T3-T4) for all samples and subsequently decreases in the last sampling times. Additionally, photosynthetic pigments values appear to be higher in fruits from the plants irrigated at 80% during the early stages of storage, reaching a maximum peak at T3 and then declining in the last two sampling points (T5–T6). In contrast, fruits from plants irrigated at 60% consistently show an initial lower chlorophyll levels compared to other samples (T0 and T1), but Chl concentration remains consistent in the final samplings T5 and T6, suggesting a possible physiological response to water stress. To confirm that "regulated" water stress can also exhibit a positive effect on chlorophyll content, in the work by Pratima, (2018) in which is evaluated the chlorophyll stability index (CSI) of kiwifruit cultivars irrigated with different volumes of water, has been tested that the

water-deficient cultivars showed a significant increase in CSI compared to the control, suggesting greater stability to chlorophyll degradation under stress conditions. Furthermore, the initial rise in chlorophyll is common in ripening yellow kiwifruits, while the later decline may result from natural chlorophyll degradation and shifts in metabolic processes. (Goffi et al., 2019; Barone et al., 2021).

A reduction in chlorophyll content during storage has also been verified by other kiwifruit cv's as well as different fruits (Grasso et al., 2022; Leunda et al., 2000; Pilkington et al., 2012; Yoko Fuke et al., 1985; Gao et al., 2019; Park et al., 2018).

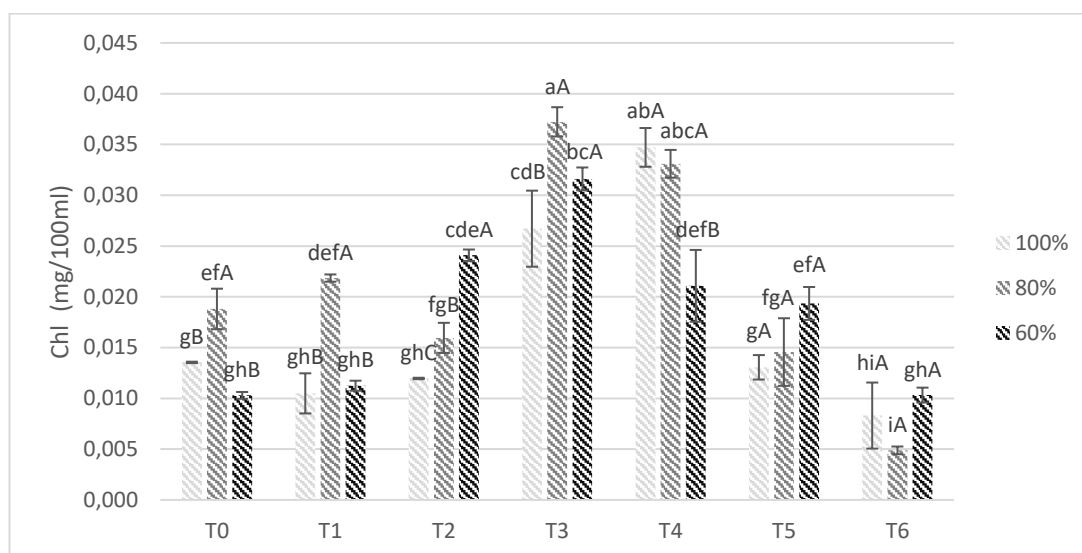


Figure 4.6. Chlorophyll in yellow kiwifruit irrigated with 100%, 80% and 60% of water at harvest (T0) and at 15 days (T1), 30 days (T2), 45 days (T3), 60 days (T4), 75 days (T5) and 90 days (T6) of cold storage at 1 ± 0.5 °C. Means depicted with the same capital letters do not show significant differences between different treatments at the same sampling time. Means depicted with the same lowercase letter are not significantly different from each other for the specific treatment. Error bars indicate standard deviations and values with different letters are significantly different ($P < 0.05$; Tukey test)

Kiwifruit is a rich source of polyphenols and in green and yellow-fleshed kiwifruit varieties ranges from 58.45 to 152 mg GAE per 100 g of fresh weight (FW) (Ma et al. 2017). In ‘Soreli’ kiwifruit treated with ozone and stored at 2° for 15 days POL content varied between 70 and 140 mg GAE per 100 g FW. The POL, FLAV and TAA content in kiwifruit, measured at various times (T0 to T6) under different irrigation levels were shown in Figure 4.7.

In our study, the POL content showed significant differences at harvest, with the highest levels observed in fruit harvested from plants irrigated at 60% water capacity (Figure 4.7a). POL levels generally increase under reduced irrigation, with

the highest values consistently observed in the 60% irrigation group. Notably, at T2, the polyphenol content in the 60% irrigation group peaks significantly, exceeding 200 mg GAE/100 g FW, indicating that a moderate water deficit positively influences polyphenol accumulation. The FLAV levels, which also show an upward trend with reduced irrigation. The 60% irrigation treatment again leads to the highest FLAV levels, particularly at T2, where there is a distinct peak of nearly 10 mg CT/100 g FW (Figure 4.7b). This suggests that water stress may enhance FLAV synthesis in the fruit. The 60% of irrigation treatment showed the highest antioxidant activity, with a marked peak at T2. This peak aligned with the trends observed for polyphenols and flavonoids content, suggesting a positive correlation between water stress and antioxidant capacity (Figure 4.7c). Our results are consistent with the findings of Jin et al. (2022), who demonstrated that four water-deficit treatment improve polyphenol and flavonoid content in tomato fruit. Several studies have demonstrated that irrigation regime increased the bioactive compound content and antioxidant activity in different apple cultivars (Ebel et al. 1993; Chenafi et al. 2016; Stefanelli et al. 2012).

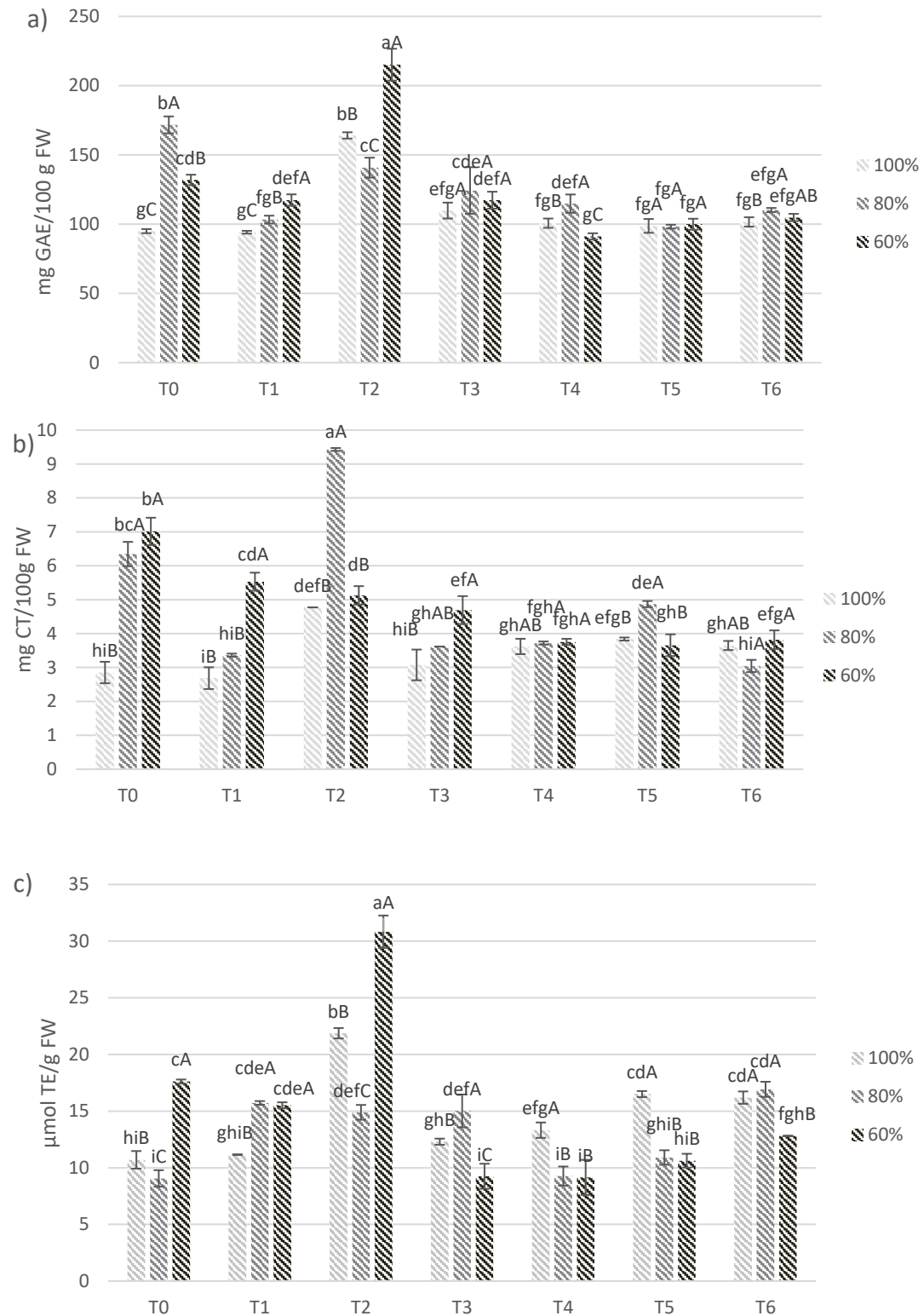


Figure 4.7. a. Polyphenol content, b. flavonoid content, and c. antioxidant activity in ‘Soreli’ kiwifruit irrigated with 100%, 80%, and 60% of water at harvest (T0) and after 15 days (T1), 30 days (T2), 45 days (T3), 60 days (T4), 75 days (T5), and 90 days (T6) of cold storage at 1 ± 0.5 °C

4.3.3. *Effect of different irrigations on antioxidant enzymatic system of 'Soreli' kiwifruit*

The activity of key antioxidant enzymes, SOD, CAT and APX, and the H₂O₂ content in 'Soreli' kiwifruit under three irrigation levels (100%, 80%, and 60%) at different storage times (T0 to T6) were reported in Table 4.2. SOD activity is highest in fruits under the 60% irrigation treatment, especially at T2, reaching the highest value (3.03 ± 0.07 $\mu\text{mol/g FW}$). SOD activity decreases gradually over storage time for all treatments, with lower levels at T6. Similar to SOD, CAT activity is highest in kiwifruit under the 60% irrigation regime with a gradual decline over storage time. Even at T6, the 60% irrigation treatment maintains higher CAT activity compared to the 100% irrigation treatment. APX activity is also highest under the 60% irrigation treatment across all time points. The activity of this enzyme decreased throughout storage; however, at the end of cold storage in the 60% irrigation treatment, it remained higher than in the other irrigation treatments (Table 4.2.). Several studies on the reduction in water supply and antioxidant enzyme activity in fruits has shown that water stress can significantly influence the enzymatic antioxidant response, activating defense mechanisms to combat oxidative stress (Ma et al., 2015; Rosalie et al. 2015; Zhang et al., 2016). Enzymes such as SOD, CAT and APX play essential roles in reducing oxidative damage by neutralizing reactive oxygen species (ROS), including H₂O₂, which increases under drought stress (Wang et al., 2007; Zhang et al., 2010). These enzymes are crucial in maintaining fruit quality, as oxidative damage can degrade cellular structures and reduce nutritional value (Pasquariello et al. 2015).

Table 4.2. Superoxide dismutase (SOD; U/g FW), catalase (CAT; $\mu\text{mol/g FW}$), ascorbate peroxidase (APX; $\mu\text{mol/g FW}$) activity and H_2O_2 (nmol/kg FW) content in kiwifruit irrigated with 100%, 80% and 60% of water at harvest (T0) and at 15 days (T1), 30 days (T2), 45 days (T3), 60 days (T4), 75 days (T5) and 90 days (T6) of cold storage at $1 \pm 0.5^\circ\text{C}$

Parameter	Treatment	T0	T1	T2	T3	T4	T5	T6
SOD	100%	1.64 $\pm$ 0.08 (klC)	1.92 $\pm$ 0.06 (hijB)	2.12 $\pm$ 0.09 (efghC)	1.88 $\pm$ 0.06 (hijkC)	1.67 $\pm$ 0.00 (jklC)	1.51 $\pm$ 0.03 (lmC)	1.31 $\pm$ 0.04 (mC)
	80%	1.98 $\pm$ 0.06 (ghiB)	2.63 $\pm$ 0.17 (bcA)	2.75 $\pm$ 0.10 (bcB)	2.28 $\pm$ 0.04 (efB)	2.09 $\pm$ 0.06 (efghB)	2.02 $\pm$ 0.05 (fghiB)	1.80 $\pm$ 0.05 (ijkB)
	60%	2.2 $\pm$ 0.05 (efgA)	2.85 $\pm$ 0.11 (abA)	3.03 $\pm$ 0.07 (aA)	2.86 $\pm$ 0.09 (abA)	2.55 $\pm$ 0.11 (cdA)	2.31 $\pm$ 0.10 (deA)	2.27 $\pm$ 0.04 (efA)
CAT	100%	5.07 $\pm$ 0.06 (cdefC)	4.85 $\pm$ 0.08 (fgC)	4.67 $\pm$ 0.05 (ghB)	4.3 $\pm$ 0.04 (ijB)	4.12 $\pm$ 0.04 (jkB)	3.57 $\pm$ 0.16 (lC)	3.03 $\pm$ 0.05 (mC)
	80%	5.84 $\pm$ 0.07 (abB)	5.17 $\pm$ 0.05 (cdB)	4.82 $\pm$ 0.09 (efgB)	4.24 $\pm$ 0.03 (ijB)	4.10 $\pm$ 0.02 (lkB)	4.04 $\pm$ 0.04 (jkB)	3.87 $\pm$ 0.07 (kB)
	60%	6.07 $\pm$ 0.05 (aA)	5.80 $\pm$ 0.08 (abA)	5.64 $\pm$ 0.10 (bA)	5.31 $\pm$ 0.20 (cA)	5.11 $\pm$ 0.03 (cdeA)	4.92 $\pm$ 0.04 (defgA)	4.51 $\pm$ 0.17 (hiA)
APX	100%	0.36 $\pm$ 0.03 (efC)	0.32 $\pm$ 0.03 (fghC)	0.29 $\pm$ 0.02 (fghiB)	0.24 $\pm$ 0.02 (ijC)	0.20 $\pm$ 0.02 (jkC)	0.17 $\pm$ 0.01 (kC)	0.13 $\pm$ 0.01 (kC)
	80%	0.47 $\pm$ 0.03 (bcB)	0.39 $\pm$ 0.02 (deB)	0.34 $\pm$ 0.02 (efgB)	0.32 $\pm$ 0.01 (fghB)	0.27 $\pm$ 0.02 (ghijB)	0.25 $\pm$ 0.03 (hijB)	0.20 $\pm$ 0.01 (jkB)
	60%	0.58 $\pm$ 0.03 (aA)	0.53 $\pm$ 0.02 (abA)	0.45 $\pm$ 0.03 (cdA)	0.40 $\pm$ 0.02 (cdeA)	0.35 $\pm$ 0.03 (efA)	0.32 $\pm$ 0.03 (fghA)	0.30 $\pm$ 0.01 (fghiA)
H_2O_2	100%	45.80 $\pm$ 1.59 (eC)	40.77 $\pm$ 0.45 (fghC)	37.97 $\pm$ 0.59 (jkC)	33.97 $\pm$ 0.32 (lmC)	33.80 $\pm$ 0.30 (lmC)	32.37 $\pm$ 0.45 (mC)	32.03 $\pm$ 0.40 (mC)
	80%	51.10 $\pm$ 0.26 (bB)	48.27 $\pm$ 1.07 (cdB)	45.77 $\pm$ 0.55 (eB)	42.93 $\pm$ 0.67 (fgB)	40.97 $\pm$ 0.21 (ghiB)	38.67 $\pm$ 0.25 (ijB)	35.83 $\pm$ 0.25 (klB)
	60%	55.23 $\pm$ 0.95 (aA)	51.90 $\pm$ 0.36 (bA)	49.90 $\pm$ 0.61 (bcA)	47.27 $\pm$ 0.86 (deA)	45.83 $\pm$ 0.85 (eA)	43.07 $\pm$ 0.74 (fA)	39.10 $\pm$ 0.56 (hijA)

Note: Capital letters indicate comparisons between different treatments of fruits at each specific time. Small letters reflect comparisons between different storage times for each sample fruit of the same treatment. According to the Tukey test ($P < 0.05$), there are no significant differences between means that share the same letter within the same column or row

In tomatoes, water deficit has been shown to enhance the activity of SOD and CAT, which helps to protect the fruit's cellular integrity and maintain its nutritional quality even under challenging environmental conditions (Ma et al., 2015; Zhang et al., 2016).

Furthermore, in pomegranate fruit deficit irrigation increased antioxidant activity, potentially due to enhance enzymatic antioxidants like SOD, CAT, and APX, protecting against oxidative damage (Nasrabadi et al. 2024). In grapes, regulated

deficit irrigation was linked to increased SOD and APX activities, helping vines manage oxidative stress and improving fruit resilience under water stress (Zhang et al. 2020) as well as water-limited conditions led to higher antioxidant enzyme activities in olives, with enzymes like CAT and APX being particularly responsive to deficit irrigation (Romero et al. 2019).

4.3.4. Effect of different irrigations on analysed features by PCA

PCA is a statistical technique that can simplify the complexity of multivariate data by reducing its dimensions, making it easier to observe trends and relationships. In this study, PCA was applied to analyze the effects of different irrigation levels on various measured characteristics in kiwifruit. By condensing the data into principal components, PCA highlights which variables are most influenced by irrigation treatments. This approach helps in visualizing how different irrigation levels correlate with changes in key physiological and biochemical traits of the fruit.

PCA plot (Figure 4.8.) illustrated the distribution of kiwifruit samples subjected to different irrigation levels (100%, 80%, and 60%) over the storage period (T0 to T6), based on principal components PC1 (41.83% of variance) and PC2 (25.1% of variance). Samples from higher irrigation levels (100%) generally cluster toward the lower right, especially in earlier time points (T0, T1), suggesting that these samples are distinct in their characteristics from those under reduced irrigation. Over time, 100% irrigation samples tend to shift along PC1, particularly between T1 and T6, likely reflecting a progressive reduction in traits like antioxidant enzyme activities. The 60% irrigation samples were distributed more towards the upper and left quadrants, indicating that these fruits retained higher values in attributes such as antioxidant enzymes (SOD, CAT) and H₂O₂ content. This distribution suggests that reduced irrigation (60%) results in a unique profile, particularly evident at T2. The middle irrigation level (80%) samples were more central but show progressive shifts toward the left, indicating a moderate response in biochemical attributes compared to the other treatments.

Overall, the PCA indicates that different irrigation levels induce distinct profiles in kiwifruit, with water deficit (60%) treatments enhancing antioxidant-related parameters, while fully irrigated samples show a consistent shift towards lower values of these parameters over time. This shift underscores the potential of moderate water stress to promote antioxidant defenses in stored kiwifruit.

Several studies have applied PCA to analyze crop characteristics under different irrigation conditions, focusing on optimizing water usage and improving yield efficiency, demonstrating how PCA can be effectively applied to optimize irrigation practices, providing valuable insights into water usage efficiency and crop performance under varying irrigation strategies (Pérez-Pérez et al. 2014; Kamchoom et al. 2024).

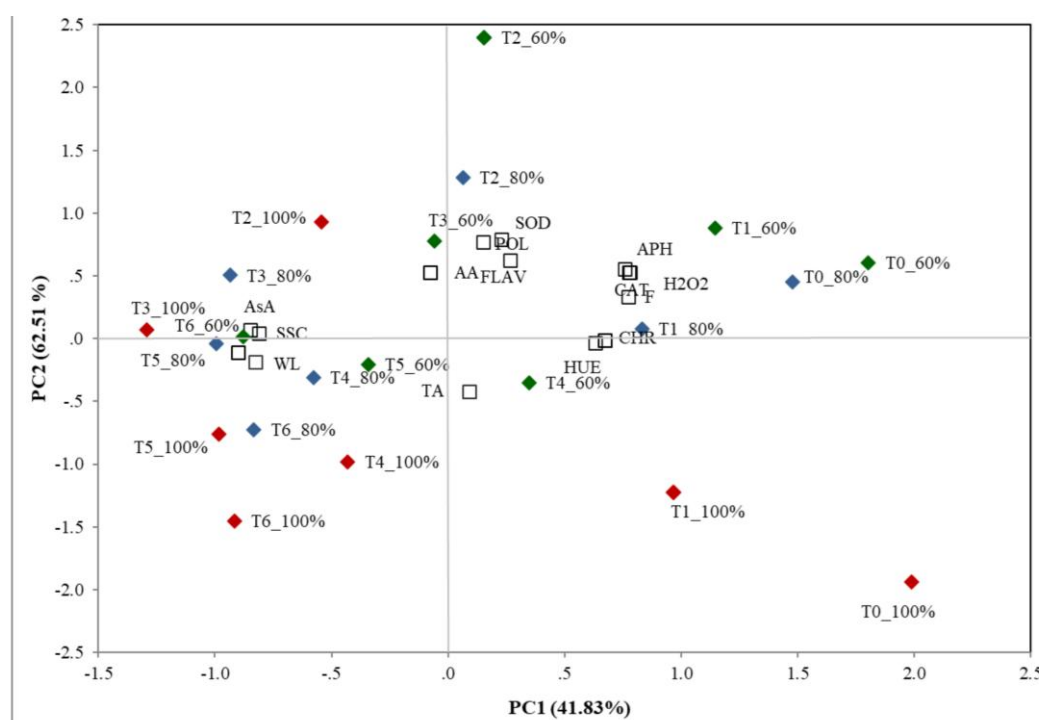


Figure 4.8. Two-dimensional principal component analysis plot of physico-chemical and antioxidant system in 'Soreli' kiwifruit irrigated with 100%, 80% and 60% of water at harvest (T0) and at 15 days (T1), 30 days (T2), 45 days (T3), 60 days (T4), 75 days (T5) and 90 days (T6) of cold storage at 1 ± 0.5 °C. (superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and H_2O_2 content (H_2O_2), ascorbic acid (AA), polyphenol content (POL) and flavonoid content (FLA), firmness (F), solide soluble content (SSC), titratable acidity (TA), Hue angle (Hue), color change (DE), Chroma (C), weight loss (WL))

4.4. Conclusion

In conclusion, the reduction of irrigation water for ‘Soreli’ kiwifruit cultivar presents considerable commercial, environmental, and as observed in our project results, qualitative advantages. Our findings indicate that a controlled reduction of pre-harvest water enhances antioxidant enzyme levels, specifically APX, SOD, and CAT, in post-harvest fruits. This suggests that reduced irrigation may activate defense mechanisms against oxidative stress.

Moreover, fruits irrigated at 60% of full water supply demonstrated increased resistance to firmness loss, higher accumulation of soluble solids, and greater color stability. Consistent with these observations, the levels of polyphenols (POL), flavonoids (FLAV), and total antioxidant activity (TAA) were higher in fruits with 60% irrigation.

This evidence suggests that an appropriate water deficit allows the preservation of certain quality attributes for longer periods, positively impacting antioxidant retention and maintaining the fruit’s quality and nutritional value during storage. These results underscore how effective water management can serve as a viable strategy to enhance post-harvest fruit quality, promoting the retention of bioactive compounds, color, and firmness while boosting the antioxidant capacity of ‘Soreli’ cultivar. Therefore, moderate irrigation emerges as a sustainable and beneficial approach to improve the quality and storability of yellow fruits.

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CHAPTER V

This article was published in Foods (MDPI) in 2025 and presents research conducted during the second and third years of my Ph.D. (2023–2025). The project was developed in collaboration with Tre Colli S.r.l., a company focused on the evaluation and enhancement of a new red kiwi cultivar. To optimize fruit quality, we investigated the effects of pre-harvest treatments with HB, aiming to improve both the coloration and anthocyanin content of the kiwis. The following sections detail the data collected and the results obtained from this study.

5. Destructive and Non-Destructive Evaluation of Anthocyanin Content and Quality Attributes in Red Kiwifruit Subjected to Plant Spray Treatment with Cis-3-Hexenyl Butyrate

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Abstract: This work evaluated red kiwifruit plants' spray treatment with cis-3-hexenyl butyrate (HB) as an inductor of some metabolic mechanisms related to fruit ripening, including an increase in anthocyanin content and the red hue color parameter. Considering their key role as ripening parameters for postharvest fruit quality and sorting assessment, the soluble solid content (SSC) and the flesh firmness penetrometer (FFP) were also measured. Treated plants received an application of 50 mM HB, administered exactly 2 and 4 weeks before the commercial harvest. At harvest time and during postharvest fruit ripening, near-infrared (NIR) spectral acquisitions were performed in order to check the feasibility of a rapid and non-destructive prediction of fruit anthocyanin content and SSC, coupled to destructive measurements and chemometric modelling. Regarding technological and chemical results, HB treatment indicates an optimum overall qualitative storage at 30 days. The fruit from treated plants is characterized by good quality parameters, including higher SSC, enhanced red hue (a^* value) and increased anthocyanin content, despite similar weight loss to

the untreated fruit. The obtained chemometric results underscore the promise and feasibility of NIRs in terms of detecting and estimating anthocyanin content and SSC in red kiwifruit, in order to pursue an evident perspective of improvement.

Keywords: red kiwifruit; NIR spectroscopy; chemometry; cis-3-hexenyl butyrate; postharvest storage

5.1. Introduction

Kiwifruit (*Actinidia chinensis*) plays a crucial role in the Italian agricultural context, serving as a key export commodity and gaining popularity among consumers due to its rich nutritional profile. Its relevant content of vitamins, antioxidants, and bioactive compounds contributes to a balanced diet and enhances consumer demand for this fruit in both local and international markets (Bakoğlu & Gunes, 2024). Globally, the leading kiwifruit producers are China (52.4%), New Zealand (13.3%) and Italy (11.5%) (Tang et al., 2024).

In recent years, consumer demand for red kiwifruit has grown, driven by its unique flavor and brightly colored pericarp (Asaidi et al., 2024). The color of the fruit's flesh has become an important focus, one which has led to the market introduction of *A. chinensis* cv “Hongyang” (cv “Red Sun”), which in turn has become the first red-fleshed kiwifruit cultivar to be grown on a commercial scale, and efforts to develop additional red-fleshed varieties are ongoing. Despite the growing interest in red-fleshed kiwifruit, little is reported in literature.

The pigmentation of kiwifruit carries significant commercial value, influencing consumer choices. These pigments contribute to their appearance and strongly appeal to consumers, leading to considerable interest in new kiwifruit varieties with red flesh (Montefiori et al., 2009).

In this context, taste, commercial value, and nutritional quality of fruit are typically correlated with total soluble solids content (SSC), key metabolites, and physical attributes like size/weight and firmness. SSC determines fruit flavor, while bioactive compounds, such as flavonoids, enhance human health. Size, weight and firmness also influence consumer preferences (Goffi et al., 2018; Goffi et al 2019; Shu et al., 2023). SSC is crucial for ripening, with acceptable levels at 12 to 15 °Brix for Hayward kiwifruit and 13 to 16 °Brix for ‘Soreli’ kiwifruit (Cipriani et al., 2018; Goffi et al., 2019; Grasso et al., 2022; Lembo et al., 2023). Although research on red kiwifruit SSC is limited, it is already known that higher SSC

correlates with a more intense red color, reaching up to 19–20 °Brix in red-fleshed varieties (Cheng et al., 2006).

Anthocyanins, part of the flavonoid family, are water-soluble pigments found in vacuoles and the primary pigments responsible for the coloration of red fruit and have been gaining significant scientific interest for several reasons (Kapoor et al., 2022). They influence the organoleptic characteristics of food, potentially affecting the ease of processing, and are linked to human health benefits due to antioxidant and anti-inflammatory properties (Khoo et al., 2017; Lu et al., 2008). These compounds are affected by environmental factors, agricultural practices and postharvest processing, making them relevant for maintaining the fruit's appeal and marketability (Asadi et al., 2024; De Pascual-Teresa et al., 2008). Enhancing anthocyanin content through biotechnology and controlled processing can improve both the nutritional and sensory qualities of food products.

Understanding the factors influencing ripening and pigmentation in red kiwifruit is essential not only for enhancing consumer appeal but also for ensuring consistent quality in the market. The conventional method used to evaluate flesh color is destructive and leads to the loss of consumable products and potential income reduction. The integration of near-infrared spectroscopy (NIR) provides valuable insights into key quality attributes, enabling growers and producers to make informed decisions about harvest timing and processing techniques. Vibrational spectroscopy in the NIR range is arguably the most widely used technique for assessing the internal quality of kiwifruit (Cevoli et al., 2024). NIR spectroscopy has been validated as a non-destructive approach for assessing SSC in Thai kiwifruit, as well as for evaluating flesh color in two commercial kiwifruit varieties: green (*Actinidia deliciosa* 'Bruno') and yellow (*A. chinensis* 'Yellow Joy'), aiding in the understanding of color development during ripening (Taticharoen et al., 2014). This is a rapid technique that requires minimal sample preparation and has proven effective for validating key quality attributes of the fruit without compromising its integrity. It offers the advantage of being easy to use and can be effectively combined with traditional analytical techniques for qualitative and quantitative analysis. When combined with multivariate data analysis, NIR can correlate spectral responses with chemical composition (Bellincontro et al., 2011), allowing for the prediction of total anthocyanins, SSC and ripeness evolution. Near infrared acoustic optically tunable filter (NIR-AOTF) spectroscopy further

enhances the non-destructive measurement of bioactive compounds, like polyphenols and anthocyanins, as already demonstrated in wine grapes (Barnaba et al., 2014). The assessment that the NIR-AOTF makes regarding significant quality and commercial attributes like sugars and weight loss (WL) is also significant. It can evaluate the optimal harvest time based on ripeness (Muganu et al., 2013) or monitor postharvest processes (Modesti et al., 2024).

Currently, the red kiwifruit on the market often exhibits poor flesh coloration, mostly limited to the central area of the fruit, with a limited radial spread. Several studies have indicated the potential use of the *cis*-3-hexenyl butyrate (HB) reagent, which, when vaporized on various horticultural plants, can mediate stomatal closure, induce resistance mechanisms in response to environmental stress and enhance fruit ripening, and can trigger an increased anthocyanin content and an improved red pigmentation in fruit, such as observed in red grapes (López-Gresa et al., 2017; López-Gresa et al., 2018; Payá et al., 2024; Payá et al., 2020).

In light of this, our study aims to investigate the potential of this natural organic compound found in the aromatic profile of strawberries in order to enhance the ripening process of red kiwifruit by promoting anthocyanin accumulation, thereby increasing the product's appeal. Additionally, we evaluate the effectiveness of NIR spectroscopy as a non-destructive method in comparison to traditional destructive techniques, allowing for real-time monitoring of the ripening process without compromising the fruit's quality.

5.2. Materials and Methods

5.2.1. Experimental Design

An experimental plot of red kiwifruit vineyards at the “Tre Colli Srl” farm, located in Velletri (Rome, Italy), was selected to study the impact of HB on the postharvest fruit quality parameters during ripening. The red kiwifruit vines from a cultivar designated BAG1 (yet to be deposited in the catalogue) were in their third year of age. The field test was conducted in the early morning hours (starting from 07:00 a.m.) on red kiwifruit plants spaced 4.0 m between rows and 3.0 m apart. Two applications of HB, diluted in distilled water at a concentration of 50 mM, were carried out using a commercial shoulder sprayer. Eight treated plants (T) and eight control plants (C) were selected on a sample basis within the row. Overall, 1000

fruit were used for postharvest study (500 fruit for each of the experimental samples). Given that the commercial harvest was carried out on 23 October 2023, the first application (application A) was made 4 weeks before commercial harvesting, while the subsequent application (application B) was performed 2 weeks before commercial harvesting. The experimental trial, including the red kiwifruit plants, the sprayer for HB treatments, and the fruit under storage conditions, is illustrated in Figure 5.1.



Figure 5.1. Experimental trial: (a) red kiwifruit plants, (b) red kiwifruit plants' spray treatment with *cis*-3-hexenyl butyrate (HB), (c) untreated (C; CK) and treated (T; 50) red kiwifruits at 0 days (T0) of cold storage at 1 °C, and (d) untreated (C; CK) and treated (T; 50) red kiwifruits at 30 days (T2) of cold storage at 1 °C

5.2.2. *Kiwifruit storage conditions*

After harvesting, the fruit—the average fruit weight at harvest was between 100–130 g with no significant variation between the two treatments—were transported to the Department for Innovation in Biological, Agro-Food and Forest Systems (DIBAF) laboratory (University of Tuscia, Viterbo, Italy), where they were selected for uniformity of size, appearance and absence of defects and diseases. Then, these fruits were packed in single-layer trays and, after 24 h of storage at room

temperature ($20\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$) (curing procedure), were cooled to $1 \pm 0.5\text{ }^{\circ}\text{C}$ with a relative humidity (RH) of $85\% \pm 5\%$ in a normal atmosphere, using an ethylene absorber in the storage rooms. All analyses were conducted at each sampling time, including harvest time (T0) and interval time of 15 days during cold storage: T1 (15 days), T2 (30 days), T3 (45 days) and T4 (60 days).

5.2.3. *Physical and Chemical Parameters*

At T0 and during each sampling time, twenty-five untreated fruits and twenty-five treated fruits were weighed using a digital balance (Adam Equipment Co., Ltd., Milton Keynes, UK) to monitor WL (%) during cold storage, and twenty-five fruits for each sample type were analysed to evaluate the technological parameters (SSC, flesh color, and flesh firmness) over time. Anthocyanin content was analysed in triplicate for each sample type at each sampling time considered.

The SSC of the fresh kiwifruit juice was measured using a digital refractometer (ATAGO, Palette PR-32, Tokyo, Japan) and expressed as $^{\circ}\text{Brix}$.

Flesh color was evaluated on peeled fruit using a Minolta colorimeter (Minolta C2500; Konica Minolta, Ramsey, NY, USA) to assess the chromaticity values via L^* (Lightness) and a^* (green to red) parameters. The hue angle (h^*) was calculated from a^* and b^* values, as reported by Mcguire (1992).

A flesh firmness penetrometer (FFP) test (Mod. 53205; TR Turoni snc, Forlì, Italy) was assessed by a destructive method by removing a 1 mm thick disc of skin from the equatorial section of each fruit. The results were expressed as Kg cm^{-2} .

For the extraction of anthocyanins, the protocol of Bongiorni et al. (2022) was employed with slight modifications. One gram of flesh fruit was taken from the reddest part of each kiwifruit sample and homogenized in 12 mL of solution composed of methanol/distilled water/hydrochloric acid (36%) in a vol ratio of 50/49/1. The samples were left for 48 h at $4\text{ }^{\circ}\text{C}$ in Teflon-shielded containers in order to prevent the degradation of the extracted anthocyanins. The anthocyanin content was determined by a spectrophotometer (Perkin Elmer Instruments Ltd., Seer Green, Beaconsfield, U.K) reading the absorbance (Abs) of the solution at a wavelength of 535 nm. Subsequently, to calculate the relative concentration of the solution, it was compared with a cyanidin-glucoside standard purchased by Extrasynthese (Genay, France) according to the following formula:

$$y = 0.2251 + 14,873 * Abs$$

The obtained values were then converted to $\mu\text{g g}^{-1}$ of fresh weight (FW).

5.2.4. AOTF-NIR spectra acquisition

A Luminar 5030 miniature portable NIR analyzer (Brimrose Corporation, Baltimore, MD, USA), based on the AOTF-NIR principle, was used for spectral acquisition (Barnaba et al., 2014). This is a portable device that can be used directly in the field, although in this reported experimental trial, the spectral measurements were conducted under laboratory conditions. Spectra detections were carried out in the range of 1100–2300 nm, with wavelength increments of 2 nm, on each intact fruit through contact between the external gun of the NIR device and the equatorial part of the fruit epicarp, using the diffuse reflectance method of acquisition. Ten fruits for each set of treated (T) and untreated (C) samples at each storage time (from T0 to T4) were spectrally measured by obtaining four hundred spectral readings for each class ($n = 400$), recorded in transmission mode and then averaged for successive chemometric computations.

5.2.5. Chemometric processing

Spectral performance was tested in the construction of PLS-type regressive models for the predictive evaluation of different destructive parameters under assessment (in detail, anthocyanins and SSC detected on the same fruit, spectrally detected as described before). The spectral data were used as predictor variables (X-block), while the chemical data were used as dependent variables (Y-block). These were then coupled when building up the models. The spectra were preliminarily transformed into absorbance ($\log 1/T$) from the original transmittance units and different pre-treatment filters were tested to identify the best performing ones; in detail, standard normal variate (SNV), Savitzky–Golay filter (1st derivative and 15 points of smoothing), and multiplicative scatter correction (MSC) pre-treatments were tested. The models obtained were cross-validated using the leave-one-out method. The accuracy of the performed PLS models was described by the coefficient of determination in calibration (R^2_{cal}) and cross-validation (R^2_{cv}), the root mean square error of calibration (RMSEC) and the root mean square error of cross-validation (RMSECV).

5.2.6. Statistical Analyses

For chemical and technological analyses, the results are expressed as the means $\pm$ standard error (SE). Statistical comparisons between different storage times of fruit from different orchard treatments were conducted using the analysis of variance (ANOVA). Tukey's test, calculated at a 5% significance level, was employed to assess differences between means. Significance was established at a p-value < 0.05 , and significant differences are indicated by different letters. To ensure comparability and mitigate the effects of varying measurement scales, the data were normalized prior to multivariate analysis using RStudio (R version 4.2.3) (Team, 2023). In detail, a principal component analysis (PCA) was performed on the dataset where each row corresponded to an individual sample of red kiwifruit (either T or C) taken at different storage times, while each column represented a specific variable under analysis.

Multivariate analyses for NIR analyses were performed, using Matlab R2013a (MathWorks®, Natick, MA, USA) and PLS Toolbox (Eigenvector Research, Inc., Manson, WA, USA).

5.3. Results and Discussion

5.3.1. *Cis-3-Hexenyl Butyrate Efficacy on Physical and Chemical Parameters and Anthocyanin Content*

As already observed by numerous studies, a gradual increase of WL over the course of storage is generally observed in all types of kiwifruits (Grasso et al 2022; Huang et al., 2017; Lembo et al., 2023). As shown in Table 5.1. an increase in WL over time for both T and C red kiwifruits has been observed, with no significant differences between samples at each storage time. At the end of storage, we reached about 4–5% of WL. Results are in line with Asadi et al. (2024) on red flesh kiwifruit. In our experiment, both samples showed an increase in SSC (Brix) over time (Table 5.1.).

Table 5.1. Physicochemical parameters (WL, weight loss; SSC, soluble solid content; FFP, flesh firmness penetrometer) of treated (T) and untreated (C) red kiwifruits at 0 days (T0), 15 days (T1), 30 days (T2), 45 days (T3) and 60 days (T4) of cold storage at 1 °C

Parameter	Treatments	T0	T1	T2	T3	T4
WL (%)	T	-	1.01 ± 0.09 (cdA)	2.73 ± 0.40 (cdbA)	3.22 ± 0.20 (abA)	4.06 ± 0.41 (aA)
	C	-	1.79 ± 0.44 (dA)	2.7 ± 0.41 (bcdA)	4.39 ± 0.60 (abA)	4.92 ± 0.39 (aA)
SSC (°Brix)	T	13.38 ± 0.74 (deA)	15.80 ± 0.70 (bcA)	18.66 ± 0.32 (aA)	18.70 ± 0.15 (aA)	18.80 ± 0.33 (aA)
	C	12.06 ± 0.56 (eA)	14.52 ± 0.27 (cdA)	17.58 ± 0.14 (abB)	17.38 ± 0.14 (abB)	18.46 ± 0.23 (aA)
FFP (Kg cm ⁻²)	T	7.12 ± 0.30 (cB)	5.22 ± 0.37 (dB)	3.08 ± 0.12 (deB)	2.47 ± 0.50 (eB)	2.12 ± 0.30 (eA)
	C	10.95 ± 0.42 (aA)	8.21 ± 0.18 (abA)	6.04 ± 0.17 (bA)	4.07 ± 0.55 (bcA)	2.13 ± 0.23 (eA)

Note: Capital letters indicate comparisons between different treatments of fruits at each specific time. Small letters reflect comparisons between different storage times for each sample fruit of the same treatment. According to the Tukey test ($P < 0.05$), there are no significant differences between means that share the same letter within the same column or row

During storage, the C sample exhibited a slower increase in °Brix compared with the T sample, reaching the maximum at the last sampling time (T4) (18.46 ± 0.23), whereas HB-treated samples show their maximum sugar content starting from T2 (18.66 ± 0.32). Obtained results agree with sugar concentrations observed by Cheng et al. (2006), who detected, in red kiwifruits, °Brix values ranging from 14.9 to 19.6. Our results are also in line with Asadi et al. (2024) after 60 days of storage of different red flesh kiwifruit cultivars (17–20 °Brix of SSC reached).

This suggests that HB treatment has a beneficial effect in maintaining SSC levels during storage, as has already been demonstrated in grape berries, where the fruit of HB-treated plants at 50 mM showed significant differences in sugar contents compared with berries deriving from untreated plants (Payá et al., 2020).

FFP decreased for all harvested fruit during cold storage (Table 5.1.). Nevertheless, it was observed that the firmness of HB-treated samples decreased significantly starting from T2, according to the SSC data. These highest values demonstrate the efficacy of HB-treatment in accelerating the ripening (López-Gresa et al., 2017; Payà et al., 2024; Payá et al., 2020). However, at T4, both samples reached the same values, according to data of Asadi et al. (2024).

Results underline that HB treatment accelerates the ripening of red kiwifruits, which is evidenced by a quicker increase in SSC and a more rapid softening compared

with untreated samples. However, though HB treatment speeds up these processes, the final WL and FFP values of T and C fruits are similar after extended storage. This appears to be aligned with what has been observed in grapefruit storage (Payà et al., 2020). Overall, these data show that red kiwifruits treated with HB are characterized by optimal quality attributes for consumption already at T2, compared with C, which has similar values only at T4.

For the color parameters of red kiwifruits, a decrease of L* value in all of the samples was observed throughout the storage, with C samples showing higher L* values than the T ones (Table 5.2.). This difference can be attributed to the HB treatment which, in turn, may have induced a greater intensity of red coloration in the fruit, potentially to the detriment of the L* value. The decrease in the L* parameter observed during the fruit ripening in both samples can be associated with the enhancement of a deeper red hue, basically due to the accumulation of pigments. This observation agrees with the results obtained by Ferrer et al. (2005) on the L* values of peach fruit, where peaches of more reddish varieties had shown lower lightness values than yellow peach fruit.

Table 5.2. Color parameters (L*, lightness; h*, hue angle; a*, Green-Red axis) of treated (T) and untreated (C) red kiwifruits at 0 days (T0), 15 days (T1), 30 days (T2), 45 days (T3) and 60 days (T4) of cold storage at 1 °C

Parameter	Treatments	T0	T1	T2	T3	T4
L*	T	54.66 ± 1.82 (bcB)	50.32 ± 2.32 (dB)	42.61 ± 0.82 (efB)	45.53 ± 1.45 (eB)	45.55 ± 1.60 (eB)
	C	65.20 ± 1.50 (aA)	58.85 ± 1.78 (abcA)	52.13 ± 1.80 (bcA)	52.06 ± 0.60 (bcA)	52.90 ± 0.39 (bcA)
h*	T	54.98 ± 2.96 (bcB)	62.66 ± 4.05 (abA)	48.09 ± 2.22 (cB)	70.45 ± 1.90 (aA)	62.84 ± 4.27 (abA)
	C	73.60 ± 3.44 (aA)	70.63 ± 1.20 (aA)	74.19 ± 2.04 (aA)	71.06 ± 1.70 (aA)	65.89 ± 2.96 (abA)
a*	T	7.32 ± 0.71 (bcdA)	9.79 ± 0.43 (bA)	14.98 ± 0.56 (aA)	8.86 ± 0.23 (bA)	9.27 ± 0.77 (bA)
	C	4.55 ± 1.30 (cdB)	6.46 ± 1.24 (bcdA)	3.80 ± 1.11 (dB)	7.97 ± 0.21 (bA)	8.39 ± 0.26 (bA)

Note: Capital letters indicate comparisons between different treatments of fruits at each specific time. Small letters reflect comparisons between different storage times for each sample fruit of the same treatment. According to the Tukey test ($P < 0.05$), there are no significant differences between means that share the same letter within the same column or row

In addition, the h* values of the fruit flesh demonstrate variability across the sampling time points, as can be observed in Table 5.2. At T0, the treated red kiwifruits exhibit a hue angle of 54.97, which is significantly lower than the

untreated fruit equal to 73.6. This difference suggests that the treated fruit may have a different color profile at harvest. Specifically, the lowest h^* value recorded for treated samples is 48.09 at T2, indicating a peak in red intensity for treated samples, as observed by Ferrer et al. (2005) in peaches, where the h^* value decreased during fruit ripening in relation to the increase in red-orange coloration. Obtained data indicate that the treatment influences the h^* of red kiwifruits, particularly in the early stages of ripening. In parallel, this could be indicative of slower color development observed in untreated fruit. In fact, it is possible to observe that T red kiwifruits show a^* value of 7.32 at the harvest time, indicating a color leaning towards red, while C fruit have a value of 4.54, due to a less red intensity. During ripening, at T2 sampling time, treated fruit reach their maximum a^* value at 14.98 (Table 5.2.), signaling a predominance of red color, while untreated fruit drop, confirming the results already observed for h^* values.

This parameter was analysed in red kiwifruits by Peng et al. (2019) and it was estimated that during the fruit ripening, the a^* value increased.

The data related to the anthocyanin contents (Figure 5.2.) reveal a dynamic change during the storage period in both T and C red kiwifruits. In kiwifruit flesh, anthocyanin concentrations increased progressively during storage in both samples, with significantly higher concentrations in HB-treated red kiwifruits. In T sample the maximum value is reached at T2, while in untreated ones it is observed at T3 and T4. The increase in anthocyanin content at time point T2 suggests not only a competitive advantage for the flesh red color of kiwifruits appreciated by the growers and consumers, but also an obvious nutritional improvement, given the role of these compounds as natural antioxidants. This observation is consistent with previous studies, demonstrating that anthocyanin accumulation in red kiwifruit pulp is absent in fruit that is harvested early, with an average of 4 °Brix. Anthocyanins typically develop concomitantly with the fruit transitions from the color change phase to full ripening (Peng et al., 2019).

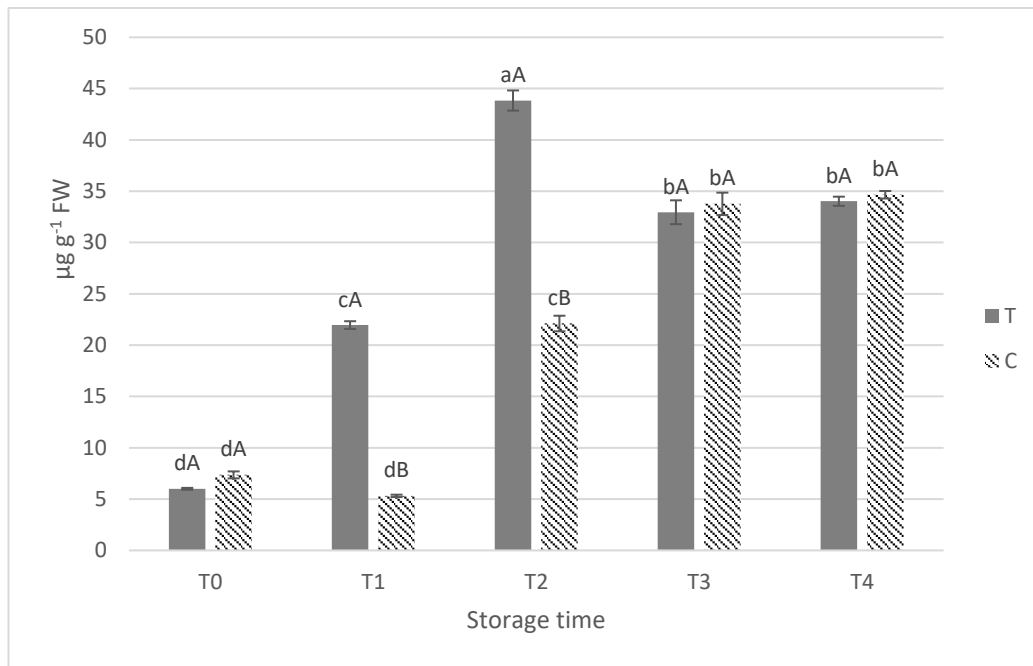


Figure 5.2. Data are for total anthocyanin content in flesh [$\mu\text{g}/\text{FW} \pm$ standard error (SE)]. Analysis of variance (ANOVA) was used to determine differences among treated and untreated samples within the same species. Different letters represent a significant difference according to Tukey's test (5% level). Different capital letters for each time indicate significant differences between kiwifruits with different treatments ($P < 0.05$), different lowercase letters indicate significant differences during storage time ($P < 0.05$)

A full and deeper understanding of the effect of HB treatment on red kiwifruit's quality, a PCA summarizing the differences, as well as relationships between T and C red kiwifruits based on the influence of all of the variables related to their composition (WL, SSC, FFP, L^* , h^* , a^* and anthocyanins) over the different storage times (from T0 to T4) has been computed. The referred results are reported in the graphical biplot included in Figure 5.3.

The sum of two principal components explains about 90% of the variance in the data ($\text{PC1} = 63.9\%$, and $\text{PC2} = 25.7\%$); original samples, after PCA computation, became the PCA scores, while the analytical variables were derived from the PCA loadings. Reported arrows represent how strongly each variable, displayed as a vector, correlates with these components and the scores segregation; the direction and length of these vectors indicate their discriminative influence and impact. The positioning of the T and C samples in the biplot shows the effect of the treatment on the composition of the red kiwifruits. Based on the separation of samples over the storage times, it can be observed that WL increases over time for both samples, as indicated by the partially positive contribution to both PC1 and PC2.

Meanwhile, FFP decreases over time by negatively affecting PC1. This is a combined observation that, as storage time progresses, T and C kiwifruits lose more weight and become softer, as expected. These trends are typically found during storage as a result of water evaporation, even if only in a limited manner when under controlled conditions, and the fruit's cell wall structure breaking down, leading to a firmness reduction (Grasso et al., 2022). Regarding color data, L^* shows a negative contribution to PC1, suggesting that, as the storage time increases, the L^* values decrease. This indicates a general loss of lightness in the red kiwifruits over time for both samples. Hue contributes positively to PC2. The positioning of the T and C samples associated with the hue vector indicates differences in color development over time. Meanwhile, the anthocyanin vector likely correlates positively with the hue vector. As anthocyanin levels increase, the hue value becomes more intense, enhancing the overall color quality of the red kiwifruits. a^* value show positive associations with PC1, and are also positively correlated between them. The positioning of the T and C samples along the a^* vector suggests how the red coloration changes over the storage period, indicating that the treatment can have an effect in enhancing the red color intensity of the fruit, also associated with a progressive increase of anthocyanin levels as the fruit ripens. The interaction between the a^* and SSC vectors in the biplot indicates that, as the kiwifruits ripen, there is a simultaneous enhancement in both red color intensity and sweetness, usually observed as an effect of ripening enhancement (Asadi et al., 2024). The SSC vector contributes positively to PC1, confirming that, as storage time progresses, SSC in both samples tends to increase. In conclusion, the biplot shows that both treatment and storage time significantly influence the physical and biochemical properties of red kiwifruits, also revealing critical changes in WL, firmness, color intensity, and sweetness. These insights emphasize the potential of treatment to enhance the overall quality of the red kiwifruits over time.

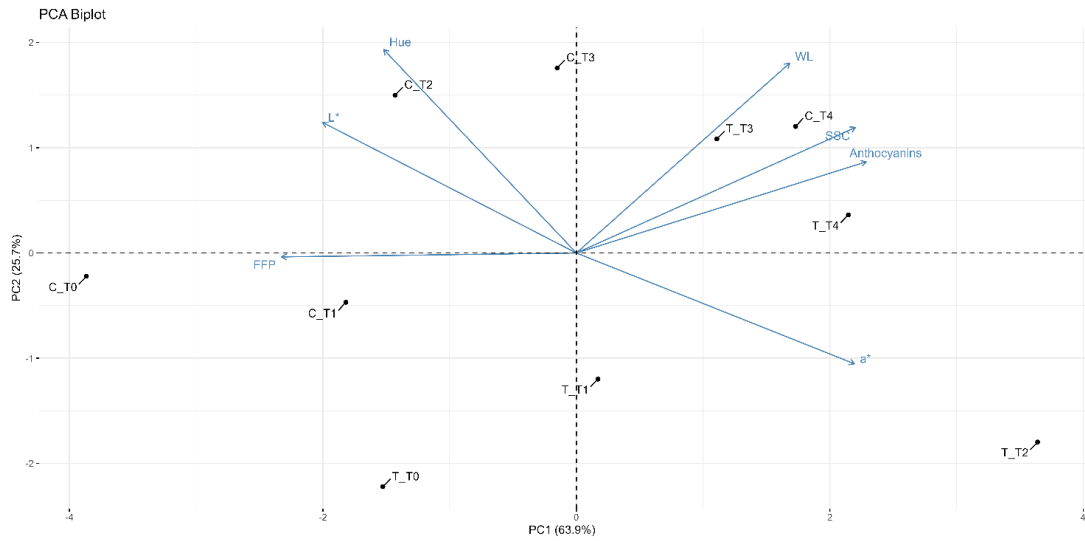


Figure 5.3. Biplot (PC1 vs. PC2) of PCA built with physical and chemical parameters (WL, weight loss; SSC, soluble solid content; FFP, flesh firmness penetrometer; L*, lightness; Hue, hue angle; a*, green–red axis; anthocyanins, anthocyanin content) for treated (T) and untreated (C) red kiwifruit samples during storage: T0 (0 days), T1 (15 days), T2 (30 days), T3 (45 days) and T4 (60 days)

5.3.2. *Nir Spectral Acquisition Models for Anthocyanins and SSC*

In parallel to the destructive evaluation of kiwifruits, NIR spectroscopy has been tested as a non-destructive and predictive tool addressed to fruit quality detection. In Figure 4, the averaged absorbance spectra (1st derivative) derived from all of the spectral kiwifruit measurements are shown; graphical representation is useful in defining the main vibrational behavior of the fruit tissue, associated with its molecular content. The first band of spectral significance observed at 1150 nm is generally assigned to the second overtone of the combination of the symmetric and asymmetric OH stretching and bending bands. Additionally, the band intercepted at around 1750 nm, which exhibits relatively low absorbance, corresponds to the first overtone of the CH stretching vibrations in CH₃, CH₂, and CH=CH groups (Bertrand & Dufour, 2006; Shenk & Westerhaus, 1991).

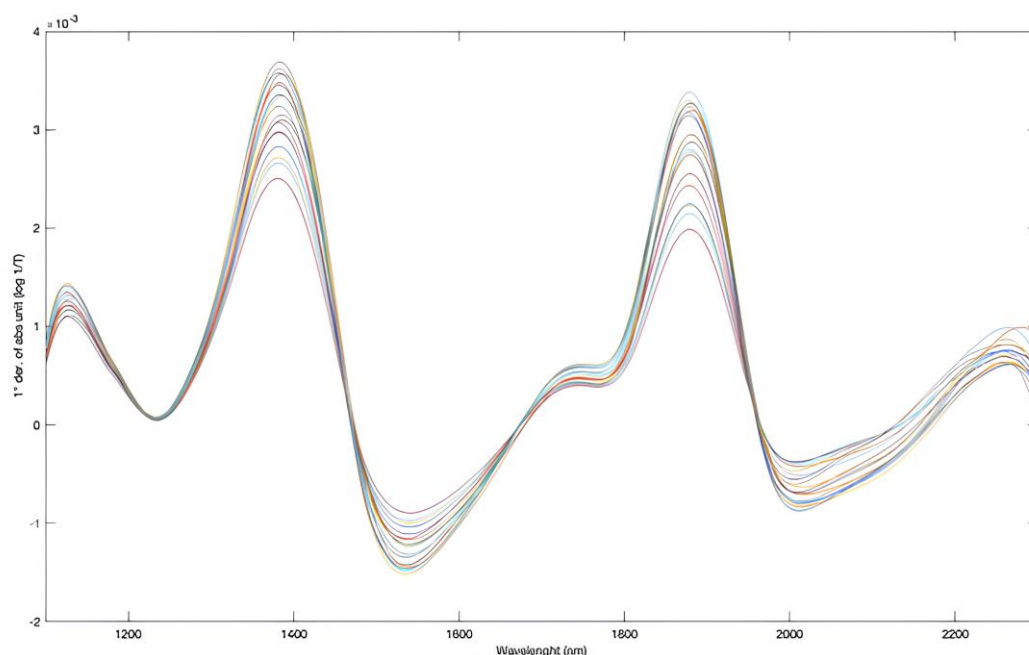


Figure 5.4. Averaged absorbance spectra (log 1/T), in 1st derivative, obtained from AOTF-NIR acquisitions. The average spectra of kiwis belonging to various sample groups are presented. The peaks with the highest absorbance correspond to the vibrational character of the resonant functional groups

The last significant band detected in the range of 2200–2260 nm refers to the resonances attributed to the combination of C-H stretching and CH₂ group in vibrational deformation (Ayvaz et al., 2022).

As typical for vegetal matrices that are spectrally measured, the absorbance spectra are characterized by two main water absorption bands at approximately 1450 nm and 1920–1950 nm (Shenk & Westerhaus, 1991). These are assigned to the first tone of the symmetrical and asymmetrical OH stretching and/or combination bands (1450 nm), as well as to the combination of the OH stretching band and OH bending band (1920–1950 nm) related to the water molecules (Bertrand & Dufour, 2006; Maeda et al., 1995).

The same detected spectra were used for the building up of regressive models performed by PLS chemometric approach aimed at predicting anthocyanin content in whole kiwifruits. Spectra were transformed into absorbance (log 1/T) and, in turn, treated with various chemometric filters to identify the most performant of the regressive computations.

In Table 5.3. the statistical indexes referring to the model performance for anthocyanin content are reported.

Table 5.3. Modeling performances in anthocyanin content prediction resulting from different pre-treatments applied to NIR spectra measurements. Indexes include R^2 (coefficient of determination) for calibration (R^2_{cal}) and cross-validation (R^2_{cv}), root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV), the number of latent variables (LV), and the ratio of performance to deviation (RPD)

Pre-treatment	R^2_{cal}	R^2_{cv}	RMSEC	RMSECV	LV	RPD
-	0.964	0.594	2.5452	8.7862	6	1.56
ABS	0.868	0.754	4.8413	6.6595	4	2.05
SNV	0.986	0.871	1.5969	4.8304	7	2.83
SG derivatives 1 [^]	0.986	0.860	1.5636	5.0582	5	2.70
MSC + autoscale	0.953	0.803	2.8866	5.9376	5	2.30

The best result was obtained by applying a standard normal variate (SNV) filtering, before PLS computation. This is a very efficient statistical filtering when reducing the within- class variance, as described by Barnes et al. (1989). The obtained model shows coefficients of determination of 0.986 and 0.860 in calibration (R^2_{cal}) and cross-validation (R^2_{cv}), respectively. The obtained RMSEC is equal to 1.60, and the RMSECV to 4.83 in calibration (RMSEC and cross-validation, respectively) while seven latent variables (LVs) minimize the predicting error (Table 5.3.). Predictive performances of PLS models was assessed by the leave-one-out cross-validation method. This method is considered appropriate when a limited dataset of samples is addressed to the regressive computation. The ratio of the root means square error in prediction (RMSECV) to the dimensionality of the destructive data (SD) resulted in a ratio of performance to deviation (RPD) of 2.83. This value was found to be above the limit of good predictive performance, which some authors have estimated at 2.5, so the optimal value of the index was instead assigned as 3 (Brown et al., 2022; Nicolaï et al., 2007).

In the literature, several authors have investigated the ability of vibrational spectroscopy, in combination with chemometric statistical techniques, aimed at predicting anthocyanin content in various vegetal crops (Barnaba et al., 2014; Bellincontro et al., 2011; Cirilli et al., 2016).

However, until now, no one has investigated this application specifically in kiwifruit, even though the aptitude of NIRs has already been tested on this fruit in

order to detect other quality attributes, like firmness, soluble solids, and dry matter (Li et al., 2024). With respect to anthocyanins, although it is widely known that their absorption response is stronger in correspondence with the visible range of light spectra, significant correlations for predicting the content of these pigments have also been found in the NIR region of the electromagnetic spectrum as reported in the available literature (Johnson et al., 2023; Manzoor et al., 2022). Due to the complex interplay of absorption mechanisms and overlapping bands, establishing a direct link between the concentration of anthocyanins, but more generally of phenolic compounds, and the dimensionality of these bands is still a challenge (Ferrer-Gallego et al., 2022).

Mariani et al. (2015), working on the prediction of the total anthocyanin content with NIRs on jaboticaba fruit, identified the spectral regions between 1232–1279 nm, 1320–1522 nm, 1792–2009 nm as the largest contributors of the spectra to regression modeling. In the present study, in terms of the spectra contribution to PLS modeling, correlation peaks were found in the region between 1156–1180 nm, at 1343 nm, and at 1888 nm.

On the other hand, significant spectra contribution to PLS modeling for SSC detection was found in correspondence with the regions between 1100–1250 nm and 1850–1950 nm, in line with what was reported by other authors. In a recent study, Cevoli et al. (2024), working with an FT-NIR device on the variety *A. chinensis* (Jintao), found a spectral significance in the region of the spectrum between 1850 and 2000 nm. According to McGlone & Kawano (1998), these portions of the light spectrum are attributed to the vibrational response of the glucidic component of the matrix.

In Table 5.4, the results in terms of model performance for SSC are reported. The best results were achieved by model computation where spectral information was transformed into absorbance, and no chemometric filters were applied. The performed model shows coefficients of determination of 0.888 and 0.784 in calibration and cross-validation, respectively. The mean square error is of 0.77 and 1.07 in calibration (R^2_c) and cross-validation (R^2_{cv}), respectively, while six latent variables (LVs) minimize the predicting error. RPD is equal to 2.19, just below the value of good predictive performance. Generally speaking, to calibrate and validate NIR as a viable method for predicting soluble solids content in fruit, a wide range of work is required to ensure that the calibration is robust (Nicolai et al.,

2007; Shenk et al., 1991). In the experimental trial here proposed, the spread of data referring to the soluble solids content varies between 11.30 and 19.30 °Brix; however, 60% of cases fall in the range of 17–19 °Brix. The results are therefore not optimally distributed, and the model is calibrated using a narrower range than if the data were normally distributed between the minimum and maximum values. This distribution, however, influences the RPD value, resulting in a lower index result; for more robust and significant results a larger dataset for both spectra and measured SSC is desirable. Nevertheless, performed results indicate a very good perspective, and they confirm the feasibility of the NIR technique for the goal of estimating quality attributes in red kiwifruits, considering further in-depth analyses and model improvement.

Table 5.4. Modeling performances in total soluble solids content (SSC) detection resulting from different pre-treatments applied to NIR spectra detections correlated with total soluble solids content (SSC). Indexes include coefficient of determination (R^2) in calibration (R^2_{cal}) and cross-validation (R^2_{cv}), root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV), the number of latent variables (LV), and the ratio of performance to deviation (RPD)

Pre-treatment	R^2_{cal}	R^2_{cv}	RMSEC	RMSECV	LV	RPD
-	0.859	0.692	0.8616	0.2966	5	1.81
ABS	0.888	0.784	0.7671	1.0746	6	2.19
SNV	0.872	0.776	0.8219	1.0939	5	2.15
SG derivatives 1 [^]	0.914	0.740	0.6707	1.1932	4	1.97
MSC + autoscale	0.941	0.713	0.555	1.278	8	1.84

5.4. Conclusions

In conclusion, the data from the study on red kiwifruits treated with HB reveal several important correlations between WL, sugar content (°Brix), firmness, color parameters (L^* , a^* , h), and anthocyanin accumulation during storage. These parameters are all closely linked to the ripening process and demonstrate that the HB treatment significantly impacts the fruit's ripening dynamics, possibly accelerating the process compared with untreated fruit. Color changes observed during storage provide further insights into the effect of HB treatment. Treated fruit showed lower L^* values and lower h^* values, correlating with higher a^* values, which signify a more intense red coloration. This suggests that HB treatment may

accelerate the biosynthesis of anthocyanins, also enhancing the visual appeal of the fruit earlier in storage.

Moreover, the obtained results highlight the feasibility of NIR technology in discriminating the presence and concentration of anthocyanins and SSC. The combined insights from HB treatment and NIR spectroscopy underscore their potential for improving kiwifruit quality, offering a background for further studies and practical applications in commercial settings. In conclusion, this preliminary study suggests the promising effect of HB treatment on the ripening quality parameters of red kiwifruit (namely on anthocyanins). With the goal to confirm the obtained data, to better elucidate the treatment effects on the plant's defense mechanisms and fruit ripening, and finally to explore a possible commercial application, we are considering a future investigation anticipating the timing of the in-field plant's treatments at lower HB concentrations.

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CHAPTER VI

This article is currently being written and presents analyses conducted during the second and third years of my Ph.D. research (2023-2025). The study focuses on evaluating the antioxidant activity of red kiwifruits treated with HB, aiming to determine whether this treatment, as previously demonstrated in Chapter IV, affects fruit ripening and nutritional composition.

6. The Role of Cis-3-Hexenyl Butyrate (HB) in Enhancing Ripening Parameters, Pigmentation, and Antioxidant Content in Red Kiwifruit

6.1. Introduction

Kiwifruit, the edible berry of climbing woody vines in the *Actinidia* genus, is highly nutritious and rich in essential nutrients. This fruit is an excellent source of vitamins A, C, E, and K, folate, carbohydrates, natural sugars, minerals, amino acids, proteins, carotenoids, and beneficial fatty acids like linoleic acid (Pinto, 2018). The content of these nutrients varies depending on the ripening stage and the kiwifruit variety (Huang, 2016; Liu et al., 2019).

Currently, more than seventy species of kiwifruit exist worldwide, with *Actinidia chinensis* being one of the most commercially significant (Costa et al., 2017). Among the commercially available varieties, kiwifruit is primarily classified into three main flesh colors, green, red, and yellow, which account for about 85%, 8%, and 7% of the total global cultivated area, respectively (Liu et al. 2019; (Black et al., 2012; Goffi et al., 2021; Gucci et al., 1996).

The presence of red flesh is primarily observed in *Actinidia Lindl* (Montefiori et al., 2009), *Actinidia arguta* (Zuo et al., 2012) and *Actinidia melanandra* (Pinto, 2018). Within the genus, there is significant variation in vine and fruit traits, such as skin and flesh color. Red pigments may be present in all fruit tissues or confined to specific areas, such as a blush on the skin, the inner pericarp region of the fruit, or only a portion of it depending on the species (Montefiori et al., 2009). Usually, this fruit exhibits a striking color contrast, with a yellow or green outer pericarp, a vibrant red inner pericarp, and a white central core. As for the skin of this genus, it is usually thinner and smoother, with a more reddish or even purplish hue in some species (Asadi et al., 2024).

Red flesh kiwifruit is known for its excellent flavor, and it is characterized by a very sweet and aromatic flavor with low acidity, exhibiting tropical notes.

Like other kiwifruit varieties, it is rich in vitamin C, dietary fiber, and antioxidants, particularly anthocyanins, which are concentrated in the red-colored portion of the flesh (Pandey & Tripathi, 2014). This fruit is highly appealing to consumers, driving significant interest in breeding new varieties with red skin and/or red flesh, coloration induced by anthocyanin accumulation (Montefiori et al., 2009). Anthocyanins are a class of water-soluble pigments belonging to the flavonoid family. In plants, they play a crucial role in protection against ultraviolet radiation before chlorophyll production begins. These compounds exhibit strong antioxidant activity by neutralizing oxidants such as molecular oxygen and free radicals (Alappat & Alappat, 2020; Khoo et al., 2017). As a result, anthocyanins are linked to various health benefits, including anti-inflammatory properties and protection against cellular damage, aging processes, and carcinogenic modifications (Alappat & Alappat, 2020; Li-Shu Wang & Gary D. Stoner, 2008).

Anthocyanins are part of the non-enzymatic antioxidant system in fruits (Stintzing et al., 2002). In addition, research has identified various antioxidant enzymes that actively neutralize free radicals within the fruit. These enzymes, together with other phenolic compounds, work synergistically to mitigate oxidative stress, thereby enhancing fruit protection and preserving quality (Boland MJ, 2013; Goffi et al., 2020; Zhu et al., 2008).

Anthocyanins are part of the non-enzymatic antioxidant system in fruits (Stintzing et al., 2002). In addition, several studies have identified an enzymatic antioxidant system that actively neutralize free radicals within the fruit. These enzymes, together with other phenolic compounds, work synergistically to mitigate oxidative stress, thereby enhancing fruit protection and preserving quality (Boland MJ, 2013; Goffi et al., 2020; Zhu et al., 2008). Among these, key antioxidant enzymes such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), and peroxidase (POD; EC1.11.1.7). play a vital role in scavenging reactive oxygen species (ROS) in plants (Zhu et al., 2008). Most red-fleshed kiwifruits contain higher amounts of total phenolics and anthocyanins, as well as high activities of antioxidant enzymes such as SOD, CAT and POD. In the red kiwifruit, these enzymes, together with the high content of anthocyanins and phenolic compounds, may work in synergistically to further enhance the fruit's defense against oxidative stress (Liu et al., 2019).

In recent years, consumer interest in red kiwifruit has grown significantly, driven by its distinctive characteristics. Its exceptionally sweet flavor, vibrant flesh color and antioxidant properties make it highly appealing in the market (Asadi et al., 2024). However, many varieties of red-fleshed kiwifruit have a weak pigmentation, primarily confined to the central region of the flesh, with minimal radial distribution. Several studies have shown that the reagent *cis*-3-hexenyl butyrate (HB) is able to increase the anthocyanin content in various fruits (Lembo et al., 2025; Payá et al., 2020). HB is an ester widely used in the food industry and fragrance production due to its fruity notes. It can be used as a flavor enhancer in fruits, contributing to their aromatic profile (de Morais et al., 2023). Due to its ability to induce stomatal closure and its involvement in the expression of genes regulating ethylene production, this compound is an ideal candidate for managing fruit ripening processes. Existing literature indicates that when vaporized onto various vegetable crops, HB can trigger stomatal closure, activate resistance mechanisms against environmental stress, and promote fruit ripening by increasing anthocyanin content and enhancing red pigmentation, as observed in red grapes (Payá et al., 2020) and red kiwifruit (Lembo et al., 2025).

This study aims to enhance the market appeal of red kiwifruit through the application of the HB compound, known for its role in fruit ripening. In red-fleshed fruits, ripening is closely associated with color development and the accumulation of antioxidant compounds. The primary objective of this research was to assess whether the HB application can intensify the desirable red coloration perceived by consumers, as well as enhance the antioxidant content, which plays a crucial role in human health.

6.2. Materials and Methods

6.2.1. Experimental Design

At the "Tre Colli S.r.l." farm (Velletri, Rome, Italy), an experimental red kiwifruit vineyard was selected to investigate the effect of *cis*-3-hexenyl butyrate on the fruit's quality parameters in the field. The red kiwifruit vines, belonging to the BAG1 cultivar (not yet catalogued), were in their third year of growth. The field trial was conducted during the early morning hours, with plants spaced 4.0 m between rows and 3.0 m between individual vines.

Two applications of *cis*-3-hexenyl butyrate (HB; 50 mM) were performed two and four weeks before harvest (October 23, 2023). Nine plants were treated with the

reagent (50 mM), while another nine untreated plants served as the control (C). The plants were randomly selected within the different rows. The treatment was applied using a commercial backpack sprayer (Figure 6.1a.) to ensure uniform coverage on the fruits, on both the upper and lower parts of the plants. The fruits were harvested on October 23, 2023 (Figure 6.1b), and placed in designated plastic crates, to prevent stacking during transportation.



Figure 6.1. Experimental trial: **(a)** Application of the *cis*-3-hexenyl butyrate treatment (50 mM) via spraying, ensuring uniform coverage on the fruits through a backpack sprayer. **(b)** Harvesting fruits at commercial time. **(c)** control (C) and treated (T) red kiwifruits at 0 days (T0) of cold storage at 1 °C

6.2.2. *Kiwifruit Storage Conditions*

After harvesting, the fruits were transported to the laboratory of the Department of Innovation in Biological, Agro-Food, and Forestry Systems (DIBAF) at the University of Tuscia (Viterbo, Italy), where they were selected for uniformity in size, appearance, and absence of defects or diseases. The fruits were then arranged in single-layer trays, and stored at room temperature ($20^{\circ}\text{C} \pm 2^{\circ}\text{C}$) for 24 hours as part of the ripening process.

Subsequently, the fruits were transferred to a cold storage room set at $1 \pm 0.5^{\circ}\text{C}$ with $85\% \pm 5\%$ relative humidity and ethylene absorbers in place. Analyses were conducted at harvest (T0) (Figure 6.1c) and after intervals of 15 days during cold storage: T1 (15 days), T2 (30 days), T3 (45 days), and T4 (60 days).

6.2.3. *Physical and chemical parameters*

At harvest (T0) and throughout storage, 25 kiwifruits were weighed using a digital balance (Adam Equipment Co., Ltd., Milton Keynes, UK). The fruit weight, expressed in grams, was recorded at each sampling point to monitor the growth progression during the field growth phase. This approach enabled the evaluation of whether the applied treatment positively influenced fruit growth, with the aim to identify any significant increases compared to the untreated fruits.

Flesh firmness was evaluated through two methods: a destructive and a non-destructive approach. The destructive method involved removing a 1 mm thick disc from the equatorial section of each kiwifruit and measuring firmness using a digital penetrometer (Model 53205; TR Turoni snc, Forlì, Italy), with results expressed in Kg cm^{-1} .

For the non-destructive method, an Instron Universal Testing Machine (Model 4301, Instron Inc., Canton, MS, USA) was used. Twenty-five fruits were compressed at a rate of

25 mm min^{-1} with a load deformation of 5 N. The same fruits were used throughout the sampling times, labeled in advance to ensure consistency. Before testing, the fruits were rewarmed to room temperature and returned to cold storage afterward. Firmness values were recorded in N mm^{-1} .

The crown color of the flesh were measured using a colorimeter (Minolta C2500; Konica Minolta, Ramsey, NY, USA), which determines color based on various chromatic parameters: L^* for lightness, a^* representing the green-to-red color spectrum, and b^* indicating the blue-to-yellow color spectrum (Lembo et al., 2023). Chroma (C^*) was calculated as reported by McGuire (Mcguire, 1992). The hue angle ($^{\circ}\text{hue}$) was calculated as $(\arctan b/a)$ (Goffi et al., 2021). The color difference (ΔE) for each sample collected was calculated. The value of ΔE expresses the difference between two colors as follows: imperceptible ($\Delta E < 1$), minimal ($1 \leq \Delta E < 2$), just perceptible ($2 \leq \Delta E < 3$), perceptible ($3 \leq \Delta E < 5$), strong difference ($5 \leq \Delta E < 12$) and different color ($\Delta E \geq 12$) (Goffi et al., 2021).

The soluble solid content (SSC), measured in °Brix, of the kiwifruits was determined from the juice extract using a digital refractometer (Palette PR-32; Atago, Tokyo, Japan). Typically, 1 °Brix corresponds to one part of solid substance, usually sucrose, in 100 parts of solution (Kleinhenz & Bumgarner, 2012).

6.2.4. *Bioactive compound levels and antioxidant efficacy*

Bioactive compounds were extracted from 2.5 g of red kiwifruit flesh using an 80% (v/v) methanol solution. The extract was then centrifuged at $14,000 \times g$ for 5 minutes, followed by filtration through Whatman No. 41 paper before being utilized for further analysis (Goffi et al., 2021).

Total polyphenol (POL) and flavonoid (FLAV) levels were assessed using the Folin–Ciocalteu and aluminum chloride colorimetric methods (Goffi et al., 2020), with 20 and 200 µl of extract, respectively. Results were expressed in mg gallic acid equivalents (GAE) per 100 g of fresh weight (FW) and mg catechin equivalents (CE) per 100 g FW.

The antioxidant capacity of the methanol extracts was evaluated using the ABTS assay (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid), following the protocol described by Ferrara et al. (2025). The ABTS radical cation solution was prepared to an initial absorbance of 0.70 at 734 nm, then combined with 5 µl of kiwifruit extract. Absorbance was measured after a 6-minute incubation. Each sample was analysed in triplicate to ensure technical accuracy. Trolox (TE) at concentrations of 4, 8, 16, and 32 µM was used as the positive control. Results were expressed as µl TE per gram of fresh weight (FW)) (Ferrara et al., 2025; Goffi et al., 2020).

6.2.5. *Enzyme isolation and activity measurements*

Total soluble proteins were extracted by resuspending frozen fruit tissue powder in an extraction buffer, with the addition of 5 mM ascorbic acid specifically for ascorbate peroxidase (APX) extraction, following the method described by Goffi et al. (2019). The homogenate underwent centrifugation at $18,000 \times g$ for 10 minutes at 4 °C, and the resulting supernatant was collected to evaluate the enzymatic activity of superoxide dismutase, catalase, and ascorbate peroxidase. Protein concentration in each crude enzyme extract was quantified using the Bradford assay (Bradford, 1976), with bovine serum albumin as the reference standard.

6.2.6. *Catalase (CAT) and ascorbate peroxidase (APX) activity*

CAT activity (EC 1.11.1.6) was assessed using the protocol outlined by Goffi et al. (2019) for 'Soreli' kiwifruit. The reaction was triggered by the addition of H_2O_2 , and the breakdown of hydrogen peroxide was tracked by monitoring the reduction in absorbance at 240 nm. Specific activity was determined as the molar rate of H_2O_2 degradation, presented as $\mu\text{mol gFW}^{-1}$.

For APX activity (EC 1.11.1.11), the method described by Goffi et al. (2019) was followed. The reaction was initiated by adding H_2O_2 , and the oxidation of ascorbic acid was measured by monitoring the decline in absorbance at 290 nm. Specific activity was calculated as the molar rate of ascorbate oxidation, expressed in $\mu\text{mol g FW}^{-1}$.

6.2.7. *Superoxide dismutase (SOD) activity*

SOD activity (EC 1.15.1.1) was determined based on its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) in the presence of riboflavin, as described by Cice et al. (2024). The reaction was monitored at 560 nm, and one unit of SOD activity was defined as the amount of enzyme needed to inhibit NBT reduction by 50% under the experimental conditions. Specific activity was expressed as U g FW^{-1} (Cice et al., 2024).

6.2.8. *Determination of Hydrogen peroxide (H_2O_2) content*

H_2O_2 content was measured by reaction mixture contained 0.1% trichloroacetic acid and 1 mol L^{-1} potassium iodide and 300 μL of crude enzyme extract, and the absorbance of the mixture was recorded at 390 nm. Results were reported as $\text{nmol H}_2\text{O}_2$ per kg of fresh weight (FW) (Cice et al., 2024).

6.2.9. *Statistical analysis*

For the physical, chemical, and enzymatic analyses, data were expressed as mean $\pm$ standard deviations. Statistical analysis was performed using two-way analysis of variance (ANOVA), followed by Tukey's test at a 5% significance level ($P < 0.05$). The statistical analysis was conducted using the DSAASTAT software integrated into EXCEL®. This approach enabled the identification of statistically significant differences between different sampling times, indicated by lowercase letters, and between different treatments at each sampling time, denoted by uppercase letters. Significant differences were indicated by different letters.

6.3. Results and discussion

6.3.1. *Effect of HB treatment on the physical and chemical parameters of red kiwifruit*

HB is a powerful, natural ripening agent commonly used in the food industries. Indeed, HB-based treatments have been tested on red kiwifruit to increase flesh pigmentation and enhance the nutritional features associated with ripening.

The weight loss (WL%) of red kiwifruit treated with HB (T) and untreated (C) did not show significant differences between the treatments, but rather over the storage period. These results were also consistent with our previous work on kiwifruit (Grasso et al., 2022; Lembo et al., 2023, 2025) but it has also been observed on various fruits such as squash (Nunes & Emond, 2007), cucumber (Al-Juhaimi et al., 2012), tomato (Javanmardi & Kubota, 2006). Our data indicate that, in accordance with the literature, both treated and untreated fruits tend to lose weight over time. Although in some sampling points (T1, T3, T4) the treated fruits appear to exhibit lower weight loss compared to the untreated fruits, no significant differences were observed between the two groups (data not shown).

Firmness of red kiwifruit, evaluated using the Instron device (Figure 6.2a), showed differences between fruits treated with HB (T) and untreated fruits (C) during the storage period. In the initial sampling points, no significant differences were observed between the two treatments. However, in the later stages of storage, treated fruits exhibited higher values compared to untreated fruits, suggesting that the HB treatment influences the ripening process by reducing the initial firmness of the fruits. The same results were obtained by analyzing the consistency using a destructive method (Figure 6.2b), in which treated fruits exhibited lower firmness compared to the untreated fruits. As confirmed by the literature, all fruits gradually lose firmness over time when stored under cold conditions (Botondi et al., 2000, 2021).

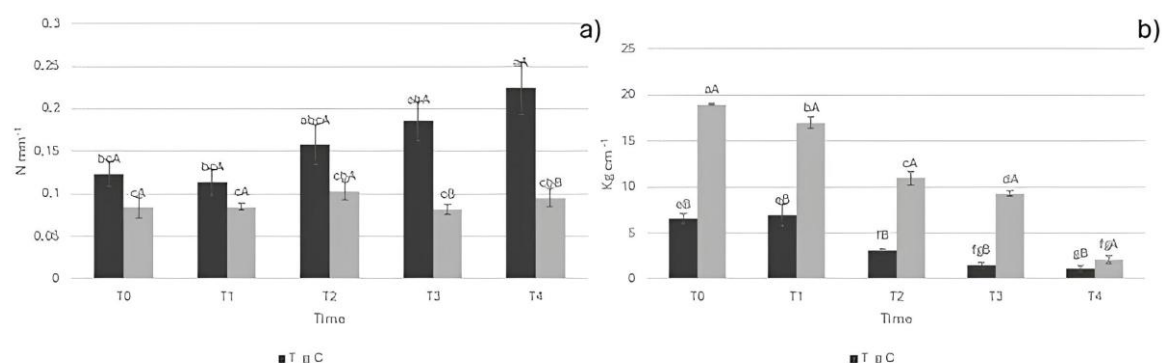


Figure 6.2. Firmness assessed by both destructive (a) and non-destructive methods (b) in fruits at harvest (T0), and after 15 days (T1), 30 days (T2), 45 days (T3) and 60 days (T4) of cold storage at 1 °C. Three technical replicates were realized for each biological sample. Significant differences between different sampling times (lowercase letters), and between different treatments at each sampling time (uppercase letters)

As for the color parameters, we focused on the parameter a^* which describes the tendency towards a red hue in the fruit crown. a^* value was found to be higher in treated fruits at all time points, and it means that the treated fruits exhibited a more intense red hue compared to the untreated ones throughout the observation period. So the treated fruits appeared redder during the initial stages of the experiment. However, a^* value became equal for both treatment at the final sampling time, it indicates that by the end of the study, there was no longer any noticeable difference in the red hue between the treated and untreated fruits (Figure 6.3.).

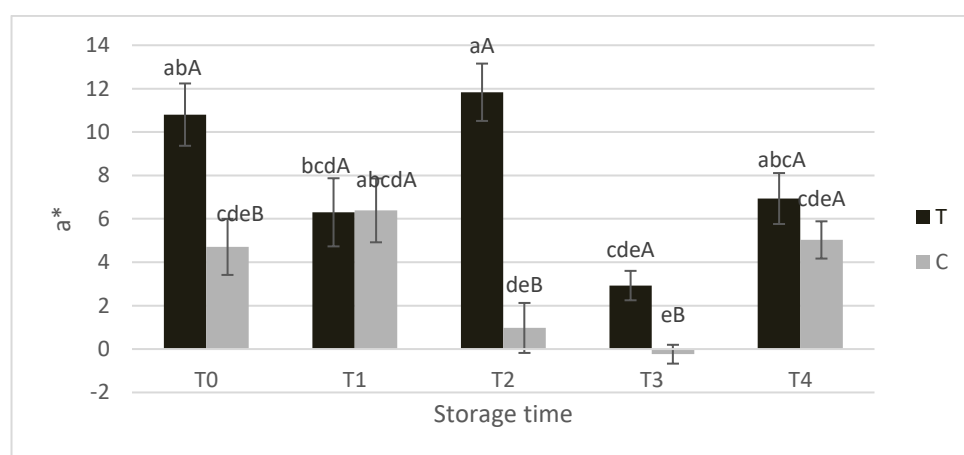


Figure 6.3. Variation of the a^* parameter (CIELAB scale) during cold storage under two experimental conditions (T and C) over different storage times (T0-T4). The a^* parameter represents the color component ranging from green ($-a^*$) to red ($+a^*$). Error bars indicate standard deviation. Different letters denote statistically significant differences between different sampling times (lowercase letters), and between different treatments at each sampling time (uppercase letters) groups ($P < 0.05$)

At the beginning of the observation, untreated fruits appeared more yellow, while the treated ones exhibited a slightly less yellowish hue. However, by the end of the storage period, b^* values were equal in both group (Table 6.1), suggesting that the yellow coloration in treated and untreated fruits became similar over time, reducing the initial color differences between them. Chroma (C^*) followed the same trend as b^* (Table 6.1), indicating that color saturation was closely linked to the yellow component (b^*). This means that at the beginning of the storage period, the untreated fruits had a higher chroma, making them appear more vibrant or saturated in yellow compared to treated fruits. Over time the chroma values also converged, suggesting a gradual equalization in overall color saturation between the two groups. While other color components like a^* (red-green) and b^* (yellow-blue) showed changes, the hue angle remained consistent throughout, suggesting the fruit's color remained in the same color family.

ΔE parameter, at the first and last sampling times, in untreated fruits deviated more from the initial color (T0) than the treated fruits. Nevertheless, during the middle of the storage period, the treated fruits experienced more significant color changes than the untreated fruits (Table 6.1). So the color of the untreated fruits fluctuated more at the start and end, while the treated fruits showed greater changes in color in the middle of the storage period, eventually stabilizing by the end.

Table 6.1. Color parameters (b^* , Yellow-Blue axis; C^* , Chroma; ΔE , Color difference) of treated (T) and untreated (C) red kiwifruits at 0 days (T0), 15 days (T1), 30 days (T2), 45 days (T3) and 60 days (T4) of cold storage at 1 °C

Parameters	Treatments	T0	T1	T2	T3	T4
b^*	T	21.77 ± 1.26 (cdB)	22.78 ± 1.18 (bcdA)	17.84 ± 1.14 (dB)	24.23 ± 1.5 (bcB)	21.10 ± 1.31 (cdA)
	C	30.43 ± 1.28 (aA)	22.31 ± 1.54 (cdA)	28.85 ± 1.79 (abA)	31.11 ± 0.52 (aA)	19.10 ± 1.39 (cdA)
C^*	T	24.74 ± 1.13 (cdB)	24.23 ± 0.85 (bcA)	21.80 ± 1.08 (cB)	24.52 ± 1.45 (bcB)	22.66 ± 0.92 (cA)
	C	31.11 ± 1.04 (aA)	23.78 ± 1.23 (cA)	29.13 ± 1.68 (abA)	31.13 ± 0.52 (aA)	20.01 ± 1.20 (cA)
ΔE	T	-	9.91 ± 1.67 (abB)	13.76 ± 0.81 (abA)	9.96 ± 1.21 (bcA)	10.3 ± 1.09 (bcB)
	C	-	16.48 ± 2.03 (aA)	9.92 ± 1.77 (bcA)	6.43 ± 0.38 (cB)	17.03 ± 1.98 (aA)

Note: Capital letters indicate comparisons between different treatments of fruit at each sampling time. Lowercase letters reflect comparisons between different storage times for each sample fruit of the same treatment. According to the Tukey test ($P < 0.05$), there are no significant differences between means that share the same letter within the same column or row

The soluble solids content, expressed as °Brix, increased during the storage period, without significant differences between treatments. This rise in SSC over time is a physiological process widely documented in the literature. As a measure of total soluble solids, primarily sugars, in plant tissues, SSC tends to increase due to various metabolic processes occurring during storage (Grasso et al., 2022; Lembo et al., 2023, 2025; Payá et al., 2020).

6.3.2. *Effect of HB treatment on Phenolic Compounds and Anthocyanin Accumulation*

Other ripening-associated parameters, such as phenolic content, were also analysed. Throughout the storage period, untreated fruits consistently exhibited higher polyphenol (POL) levels compared to treated ones, both at the beginning and the end of the experiment, both at the beginning and at the end of the storage period (Table 6.2.) However, when considering flavonoid (FLAV) content, no significant differences were observed between treated and untreated fruits. Similar findings were reported by Payà et al. (2020) in grapes treated (Payá et al., 2020).

Total anthocyanin (ACN) content was significantly higher in HB-treated plots at 50 mM, compared to non-treated plants, which properly correlates with the color development. Moreover, our findings align with previous studies by Lembo et al. (2025) and Payá et al. (2020) regarding the use of the HB reagent (Lembo et al., 2025; Payá et al., 2020).

Table 6.2. Polyphenol (POL), flavonoids (FLAV) and anthocyanins (ACN) content of treated (T) and untreated (C) red kiwifruits at harvest (T0), and after 15 days (T1), 30 days (T2), 45 days (T3) and 60 days (T4) of cold storage at 1 °C

Parameters	Treatments	T0	T1	T2	T3	T4
POL	T	198.78 ± 9.38 (dB)	252.92 ± 25.25 (bcdA)	213.64 ± 14.69 (cdA)	250.32 ± 13.65 (bcdA)	255.84 ± 8.26 (bcdB)
	C	322.42 ± 15.43 (aA)	258.93 ± 24.78 (bcA)	224.87 ± 2.81 (cdA)	262.12 ± 10.19 (bcA)	298.99 ± 7.21 (abA)
FLAV	T	7.96 ± 0.08 (cdB)	5.82 ± 0.08 (bcA)	6.35 ± 0.33 (cB)	7.28 ± 0.33 (bcB)	6.97 ± 0.37 (cA)
	C	8.12 ± 0.21 (aA)	6.80 ± 0.63 (cA)	5.24 ± 0.21 (abA)	6.02 ± 0.12 (aA)	8.07 ± 0.08 (cA)
ACN	T	9.49 ± 0.08 (bA)	13.1 ± 0.03 (aA)	7.88 ± 0.28 (dA)	8.21 ± 0.14 (cdA)	9.18 ± 0.09 (bcA)
	C	4.16 ± 0.04 (fB)	3.42 ± 0.04 (fB)	5.33 ± 1.04 (eB)	4.40 ± 0.01 (efB)	2.22 ± 0.01 (gB)

Note: Capital letters indicate comparisons between different treatments of fruit at each sampling time. Lowercase letters reflect comparisons between different storage times for each sample fruit of the same treatment. According to the Tukey test ($P < 0.05$), there are no significant differences between means that share the same letter within the same column or row

6.3.3. Effect of HB treatment on antioxidant enzymes

In Table 6.3. were shown the activity of key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), in treated (T) and untreated (C) red kiwifruits at harvest (T0) and during cold storage (T1–T4). The data indicate fluctuations in enzyme activity over time, with notable differences between treatments at certain time points. HB treatment appears to induce an initial surge in SOD activity, which is not sustained during mid-storage. In contrast, untreated fruits maintain a steadier SOD activity over time, which might suggest that HB modulates the oxidative stress response in a time-dependent manner (Table 6.3.). The treated fruits exhibit an accelerated catalase response, with a marked early increase that peaks sooner compared to the untreated fruits. However, as storage progresses, untreated fruits catch up and even surpass the treated fruits at T3, suggesting that HB treatment may prompt an earlier but transient catalase response (table 6.3.). The HB treatment appears to stabilize APX activity over the storage period, preventing the decline observed in untreated fruits. This stabilization may contribute to a more consistent antioxidant defense in the treated

samples.

HB treatment influences the enzymatic antioxidant system in red kiwifruits in a time-dependent manner. Treated fruits exhibit an early surge in both SOD and CAT activities, suggesting an immediate response to oxidative stress that may be induced by the HB reagent. However, this response is not uniformly sustained over the entire storage period, as indicated by the declines in SOD and CAT activities at mid-storage, followed by a partial recovery or stabilization later on. In contrast, untreated fruits tend to show more gradual and sustained enzyme activities.

The contrasting patterns, particularly in APX activity where treated fruits maintain steadier levels compared to the declining trend in untreated fruits, suggest that HB treatment may help in preserving a more consistent antioxidant capacity over extended storage. These dynamic changes highlight the complex interplay between ripening, oxidative stress, and the modulation of antioxidant defenses by HB treatment in red kiwifruits. This is the first study to evaluate the effect of *cis*-3-hexenyl butyrate (HB) on antioxidant enzymes, highlighting its potential as a natural ripening agent with positive effects on fruit quality and health benefits. Antioxidant enzymes, which are crucial for neutralizing reactive oxygen species (ROS), were found to respond differently to HB treatment, with significant increases in activity associated with improved fruit quality and color.

Table 6.3. Superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activity of treated (T) and untreated (C) red kiwifruits at harvest (T0), and after 15 days (T1), 30 days (T2), 45 days (T3) and 60 days (T4) of cold storage at 1 °C

Enzymes	Treatments	T0	T1	T2	T3	T4
SOD (U/g FW)	T	0.85±0.14 (deA)	1.56±0.17 (aA)	0.62±0.01 (efB)	0.32±0.14 (fB)	1.27±0.23 (abcdA)
	C	1.11±0.16 (bcdA)	1.43±0.04 (abcA)	1.52±0.10 (abA)	1.32±0.14 (abcA)	1.01±0.20 (cdeA)
CAT (µmol/g)	T	2.89±0.05 (deA)	4.39±0.62 (bcA)	8.81±1.34 (aA)	3.92±0.15 (cdB)	3.90±0.21 (cdA)
	C	2.22±0.31 (eB)	1.87±0.15 (eB)	4.55±0.37 (bcB)	5.70±0.09 (bA)	4.16±1.96 (cdeB)
APX (µmol/g)	T	0.25±0.04 (cdA)	0.36±0.02 (abA)	0.30±0.02 (bcA)	0.30±0.02 (bcA)	0.34±0.04 (abA)
	C	0.23±0.01 (dA)	0.40±0.02 (aA)	0.25±0.01 (cdB)	0.23±0.01 (dB)	0.19±0.01 (dB)

Note: Capital letters indicate comparisons between different treatments of fruit at each sampling time. Lowercase letters reflect comparisons between different storage times for each sample fruit of the same treatment. According to the Tukey test ($P < 0.05$), there are no significant differences between means that share the same letter within the same column or row

6.4. Conclusions

This preliminary study demonstrates that *cis*-3-Hexenyl Butyrate (HB) treatment significantly influences the ripening and quality of red kiwifruit. HB-treated fruits exhibited higher anthocyanin levels and increased a^* values during the early storage stages, indicating a more intense red coloration. Although the differences in red hue between treated and untreated fruits diminished by the final sampling point, the initial enhancement supports the role of HB in promoting pigmentation. Despite no significant differences in weight loss or soluble solids content (SSC) between treated and untreated fruits, the improvements in pigmentation and antioxidant enzyme activity suggest that HB treatment can positively affect the overall nutritional and sensory quality of red kiwifruit. The HB treatment induced an early surge in the activities of key antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), suggesting a prompt oxidative stress response. Furthermore, ascorbate peroxidase (APX) activity remained more consistent in treated fruits compared to the declining trend observed in untreated samples, indicating a preserved antioxidant defense. Further research is warranted to elucidate the molecular mechanisms underlying these responses and to explore the broader applicability of HB treatments across different fruit species.

6.5. Selected References

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