DISSERTATION

INFLUENCE OF ORCHARD FACTORS ON APPLE HARVEST QUALITY AND POSTHARVEST PERFORMANCE

Submitted by

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ABSTRACT

INFLUENCE OF ORCHARD FACTORS ON APPLE HARVEST QUALITY AND POSTHARVEST PERFORMANCE

Focusing on both preharvest and postharvest elements, this study investigates how crop load affects apple growth, development, and quality. A worldwide important fruit crop, apples have been grown for over two thousand years, with more than 10,000 types known. Several elements affect apple quality, including environmental factors, genetic factors, and orchard management techniques. The impacts of crop load were investigated on both physiological and metabolomic features, hence stressing its significance as a main factor of ultimate apple fruit quality. The study underlines how apple quality is affected by preharvest variables and how suitable postharvest handling can maintain this quality over storage, hence encouraging more apple consumption. Current regulation and consumer demand provide high economic value for fruits with superior sensory and nutritional qualities. Improvement of apple fruit quality is impossible postharvest. Hence, optimum apple quality at harvest and during postharvest and subsequent, consumer satisfaction, is achievable through understanding the influence of preharvest factors. A large-scale study on the effect of crop load on ‘Gala’ fruit growth and development and on harvest internal quality and maturity was performed. Hand thinning was used to achieve three crop load treatments: heavy load (978 fruit/tree), commercial load (598 fruit/tree), and light load (380 fruit/tree) that were compared to unthinned trees (1614 fruit/tree). Parameters studied were yield, fruit size during growth and development and at harvest, fruit red overcolor blush, starch index, flesh firmness (FF), soluble solids concentration (SSC), dry matter content (DMC), index of absorbance difference (I_{AD}), return bloom and fruit set (next season). Non-destructive models that utilize visual to near infrared spectroscopy (Vis-NIRS) were used to predict internal quality in terms of DMC, SSC and I_{AD}. Apple thinning decreased fruit yield, increased fruit size, DMC, and SSC, improved fruit red overcolor blush and advanced fruit maturity based on I_{AD}. However, it did not affect the starch index and FF. Vis-NIRS technology accurately estimated DMC and SSC at 729-935nm, and maturity based I_{AD}. Additionally, I_{AD} did not correlate with FF but described better fruit physiological maturity. Non-destructive technologies that predict internal fruit quality and maturity are powerful tools that provide a better understanding of the effect of pre-harvest factors on apple fruit quality. In terms of postharvest performance, fruits coming from distinct crop load management treatments were treated or not with 1-methylcyclopropene (1-MCP, 1000 $\mu l\ l^{-1}$, 24 h, 0^{o} C) an ethylene action inhibitor and known for its effect on prolonging apple fruit quality. Following pre-storage treatment or not with 1-MCP apples coming from heavy, commercial, light crop loads and unthinned trees were cold stored (0^{o}C, 95%, RH) for up to 3 or 7 months. After cold storage fruits were transferred at room temperature (20^{o}C, 90% RH) to simulate retail market conditions and apple ripening was characterized immediately at 0, 4 and 8 days. Collectively, crop load and 1-MCP significantly affected apple fruit storage performance. Fruit coming from lighter crop loads had increased levels of SSC during postharvest ripening due to higher DMC and expressed significantly lower softening rates during storage potentially because of increased carbohydrates pool (DMC and SSC) that potentially correlate with delayed deterioration. Treatment with the 1-MCP through inhibition in respiration and ethylene production blocked fruit ripening, retained higher DMC and SSC levels, and delayed softening and loss of titratable acidity

(TA) during postharvest compared to untreated fruits. These results are of high importance for the apple industry as they demonstrate that proper preharvest management could lead to robust postharvest performance.

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DEDICATION

To my parents, Fatima and Mustafa:

The ones that brought me into this world,

the ones that stood with me to achieve my education,

the ones who never gave up on me

To my husband, Youssif:

The man who encouraged me to take this path,

The one who never made me feel bad

The one that stayed by my side through thick and thin

My other half

To my kids:

The ones who brought the upmost happiness into my life

The ones who stayed with me and entertained me

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The one that never failed to check up on me daily

The one who was basically like a parent to me

The one that could read me best and had to make sure everything was okay with me before resting, regardless of the time difference

The one who stepped up

To my sisters:

They're the ones who filmed videos of me when they all got together to make sure I didn’t miss anything.

The ones that brought me joy and made me laugh even at my lowest

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CHAPTER ONE GENERAL INTRODUCTION

1. Apple economic importance

Apple (*Malus x domestica* Borkh.) is one of the four most important fruit crops worldwide and in the United States, following all citrus types, bananas, and grapes (FAO, 2023). Worldwide apple production observed an increase until 2022 (Figure 1.1.A). The United States is the third largest apple producer in the world (Figure 1.1.B), where it is currently producing 6% of global production, following China, which is producing 57% of global production, and the European Union, which is producing 13% of global production (USDA-NASS, 2024) (Figure 1.1.B). Apple production in the United States continued to increase until 2017 when the total apple production was valued $2.8 to $3.1 billion dollars (USDA-NASS, 2023). Most of the United States grow apples, however, Washington is by far the top apple producer in the country (Fig.1.1.C), contributing more than 70% of the total US apple production (Geisler, 2012). In Colorado, apples are the second largest fruit crop after peaches and the main apple production areas are centered around the towns of Cedaredge, Hotchkiss, and Paonia (Delta County) with significant organic production in those areas. Currently there are 1,200 acres producing about 4,000 tons of apples every year, which are valued at around $7.3 million (FAO, 2017, and USDA-NAAS, 2015, and 2017). The importance of apple fruit lies in its nutritional and human health benefits. Apples are an important source of essential minerals and beneficial phytochemicals such as phenols, flavonoids, and carotenoids. These phytochemicals contribute to several functions including antioxidant activity, cell signaling effects, and antiproliferative (Hyson, 2011) that play a kay role in reducing chronic diseases, cancer, asthma, diabetes, and many other health problems (Boyer et al., 2004).

2. Apple fruit quality

Fruit quality is a general notion that describes physical properties (firmness, mass, volume), mechanical (absence of defects), sensorial properties (texture, taste, and aroma), nutritional value (abundance of antioxidants and vitamins), aesthetic appearance (color, size, and shape) and food safety factors (Crisosto and Costa, 2008; Minas et al., 2018). In addition, different links of the supply chain (consumers, growers) weigh these properties differently, for example fruit growers are interested in yield, fruit size, shape, and fruit firmness, while consumers are interested in nutritional and health benefits (Vanoli and Buccheri, 2012; Musacchi, and Serra, 2018).

Apple, a member of the Rosaceae family, is a perennial fruit crop that has a long production life and requires consistent cultural management to regulate growth and development processes and optimize fruit quality (Li et al., 2015). Apple is a climacteric fruit which means that fruit ripening is orchestrated by increased ethylene synthesis both on-tree and postharvest. Additionally, physiological disorders that affect climacteric fleshy fruits during postharvest are the most highly debated problems in apple production (Watkins et al., 2006; Mattheis, 2008). Apple fruits are also known for their capacity to accumulate high levels of starch in the cortex tissue while on-tree, which is hydrolyzed to soluble sugars during postharvest storage and ripening prior to reaching consumers (Singh et al., 2017). Hence, it is important to point out that as apple quality peaks at harvest, a deeper understanding of how preharvest variables affect apple quality and how to maintain it via appropriate postharvest treatments might lead to maintaining its quality during storage and that helps increase apple fruit consumption and minimize postharvest fruit loss (Crisosto et al., 1997; Kyriacou and Rouphael, 2017; Minas et al, 2018; ; Shewa et al., 2022).

3. Orchard factors affecting harvest quality and postharvest performance

Apple harvest and postharvest quality is affected by two major agronomic factors (genotype and orchard management) and the environmental conditions (Musacchi et al, 2017) (Fig 1.2). In addition, the interaction between agronomic and environmental factors plays a significant role in apple tree physiology, productivity, and apple fruit quality. Genotype refers to cultivars and rootstocks that both can have a major impact on fruit sensory quality (Miller et al, 2005). Over the last years, apple breeding has been focused on the selection of cultivars with more skin red over-color, sweetness, and less susceptibility to sunburn (Musacchi et al, 2017). On the other hand, modern rootstock selections show improved yield, fruit quality, and limit tree vigor (Musacchi and Serra, 2016).

Environmental factors refer mainly to temperature that can affect fruit quality when high or low temperatures occur at different developmental stages. For example, high temperatures close to harvest cause watercore (Fig. 1.3) that turns to internal breakdown during storage and reduce skin red over-color (Fig 1.4). Low temperatures during bloom time cause frost rings (Fig 1.5), but low temperatures above freezing close to harvest increase skin red over-color (Fig. 1.4). It has been summarized that the effect of environmental factors like sunlight, temperature, and relative humidity is combined with soil characteristics and orchard management to determine fruit quality at harvest (Corelli-Grappadelli and Lakso, 2004; Musacchi et al., 2017). Orchard management factors that affect apple productivity and quality include irrigation, mineral nutrition, canopy architecture, pruning, fruit position in the canopy, orchard design, and orientation and crop load management.

Irrigation affects tree and fruit growth and deficit at critical developmental stages could severely impact yield and fruit size, on the other hand, over irrigation close to harvest time could cause watercore. Optimum mineral nutrition improves productivity and fruit quality, however nutrient imbalances or deficiencies could severely compromise fruit yield and quality. For example, the imbalance between Ca/N_2, K, and Mg causes bitter pit which is a physiological disorder for apples and major cause of fruit loss. In addition, boron deficiency causes corking which is a small, flushed region on the skin of the fruit above the impacted brown spot in the flesh, resulting in affected apples often appearing gnarled and pitted and distorted. The impacted tissue is typically significantly denser than the healthy tissue (Singh et al., 2019).

Canopy architecture and cropping systems affect light interception and penetration within the tree (Tustin et al., 2022). Functional light energy interception in fruit crops including apples is correlated with yield and total production dry matter (Palmer et al., 2002). However, apple cropping systems must achieve uniform light distribution with available light >50% across the entire canopy for production efficiency and optimum fruit quality. In addition, the fruit position in the canopy affects apple fruit size, ripening time and red over-color blush. Horticultural management practices and preharvest treatments can be used to enhance apple fruit quality such as pruning and training techniques, and chemical thinning for efficient crop load management, or plant growth regulator (PGR) sprays for harvest delay (Musacchi et al., 2017). Among all horticultural management strategies, the most impactful for fruit quality optimization is crop load management (Serra et al., 2016).

3.1. Genotype

3.1.1. Cultivars

Apples have been cultivated for more than two thousand years. There are about 10,000 recognized cultivars, and breeders globally release several new selections every year, yet, only a few dozen varieties are extensively grown commercially nowadays (Janick et al., 1996; and Ferree et al., 2003). The elevated production cost requires an apple cultivar to exhibit tolerance to pathogen infections, pest infestations, abundant and regular yields of commercial-grade fruit, that are suitable for postharvest handling, long-term storage, with increased tolerance to postharvest disorders and capable of stimulating substantial consumer demand. According to Iglesias et al. (2012) fruit quality is closely related to cultivar, and many quality traits are inherited. Furthermore, the quality attributes variability in certain commercially important cultivars, such as ‘Gala,’ ‘Fuji,’ ‘Golden Delicious,’ ‘Jonathan,’ ‘Rome Beauty,’ and ‘Red Delicious’ (Gorini, 1982; Iglesias et al., 2012; Musacchi et al., 2018). For example, numerous cultivars produce bi-color apples such as ‘Gala’ or ‘Fuji’ which are harvested in many picks to achieve an optimum red over-color level and price premiums particularly in temperate regions (Marsh et al., 1996; Musacchi et al., 2018). Furthermore, sunburn sensitivity varies across cultivars and severely impacts the appearance and economic returns of apple production. Severely affected fruits are discarded at harvest and moderately affected fruit experience a decline in quality during postharvest handling (Schrader et al., 2009). For example, many studies indicated a significant sensitivity of ‘Jonagold’ and ‘Granny Smith’ to sunburn, however, ‘Topaz’, ‘Cripps Pink’ and ‘Idared’ have been documented as more tolerant (Sibbett et al., 1991; Van den Ende, 1999; Schrader et al., 2003; Racsko et al., 2005; Musacchi et al., 2018). Also, cultivars significantly affect the sweetness of the fruit, as certain varieties have inherently higher soluble sugars content and lower acidity levels, thereby directly influencing the perceived sweetness from consumers. For example, Fuji apples are regarded among the sweetest, whereas 'Granny Smith' apples are characterized by their sourness (Zhang et al., 2023).

3.1.2. Rootstocks

Rootstocks represent the root section of an apple tree that is usually a cultivar or an interspecific hybrid of the *Malus* sp. that is grafted with another apple cultivar (the scion). The rootstock influences several aspects of the tree performance, while the scion dictates the apple fruit type that is going to be produced by the tree (Webster and Wertheim, 2003). In addition to the production of a robust crop, apple scion grafting onto rootstocks is the foundation of modern tree fruit production that provides many additional advantages to the growers. The advantages of apple rootstock use include:

1. Tree vigor limitation and yield efficiency: dwarfing rootstocks help the maintenance of a compact fruit tree, hence increasing planting densities and simplifying orchard management and harvesting (Van Hooijdonk et al., 2011). Using dwarfing rootstocks like M9 the entire industry was revolutionized with the adoption of High-Density Plantings (HDP). These types of vigor limiting rootstocks promote floral bud initiation and favor carbon distribution to fruit production instead of equal distribution between reproduction and vegetative growth which is the case with vigorous rootstocks (Autio et al., 2020). Hence, it is worth noting that there are three major classes of rootstock vigor: dwarf, semi-dwarf, and standard. Apple trees grafted onto dwarf rootstock such as M9, B10, B9, G41, etc. (Fig. 1.7) achieve size ranging from 15% to 45% of that of a standard size tree while semi-dwarf rootstock such as MM111which is the most common grown? to 50% to 75% the size of a seedling (Fig. 1.7) (Autio et al., 2020).

2. Disease and pest tolerance: apple rootstock selections exhibit remarkable tolerance to prevalent apple tree diseases such as *phytophthora*, replant disease, and fire blight, nematodes and aphids allow cultivation even in infected or infested soils (Marini et al., 2018). For example, in their study, Browne and Mircetich (1993) found that different rootstocks vary in their resistance depending on the type of *Phytophthora*. Rootstocks that showed resistance to *P. cactorum* were B.118, M.9, Mark, and MM.106; in contrast, Ant.313 and MM.106 were very susceptible. Mark was one of the rootstocks that was resistant to *P. cambivora*. The remaining strains were very susceptible. whereas B.118, B.9, M.7, and P.18 were in the moderate range. Outside of M.4, MM.111, Ant.313, and P.18, most of the rootstocks showed some resistance to *P. cryptogea*. In case of fire blight, Marini et al. (2000) reported that "Gala" trees on B.9 survived longer than trees on M.9 and M.26 during times of severe fire blight infection.

3. Soil adaptability: different rootstocks have been selected for their ability to perform well in thick clay soils, while others exhibit drought tolerance. M.111, a semi-dwarfing rootstock, produces sturdy, drought-resistant apple trees. M9 is a drought-tolerant rootstock that thrives in dry soil. Both rootstocks are popular because they can develop apple trees in dry, difficult conditions (Psarras and Merwin, 2000).

4. Tree precocity: apple seedlings may need up to ten years to bear commercial fruit yields; however, certain apple rootstock selections can enable the first harvest within two years of planting. The primary effects of rootstock on scions that concern orchardists include fruit bud formation, fruiting precocity, fruit set, and yield. Rootstock influences both the yield of the tree and its yield efficiency. The plants on dwarfing rootstocks M9 and M20 yielded higher cumulative production by up to 10 years of age due to their early fruiting (Jat et al., 2022).

5. Cold hardiness: cold temperatures may adversely affect fruit trees, and cold-hardy rootstocks are advantageous for those growing in colder northern areas like the Intermountain West region. To clarify, Basedow (2019) assessed rootstock hardiness to evaluate the cold resistance of various rootstocks in comparison to one another, categorizing them into broad groups from more to less cold hardy. The most cold-hardy dwarfing rootstock is M26, followed by M9 and B9. Different rootstocks have been reported to affect fruit size, scion cold hardiness, fruit skin color, soluble solids concentration, dry matter content, harvest time, storage quality, and respiration rates at harvest and during postharvest. This highlights the impact of rootstock on multiple parameters that impact fruit handling, waste, and quality for the consumers (Barritt et al., 1997).

3.2. Environmental factors

3.2.1. Light

Certainly, light is one of the most important preharvest factors associated with fruit quality and maturation that also affects fruit postharvest performance (Farcuh, 2022; Kviklys et al., 2022). Visible light within the 400–700 nm wavelength range is the primary catalyst for biomass formation through photosynthesis, hence dry matter production in apple trees has been shown to correlate with the quantity of visible light intercepted by the tree canopies (Palmer, 1989; Lakso, 1994). Robinson (2017) proposed that with an efficient apple cropping system and optimum yield, light interception should reach 70–75%. In addition, light significantly affects apple fruit quality by promoting the development of fruit skin over-color through the synthesis of anthocyanins, soluble pigments that provide the red color of fruit tissues in multiple species including apple. Apples exposed to more sunlight would exhibit a more intense red hue but at the same time excessive light may induce sunburn damage to the apple skin (Fig.1.6). High levels of light availability within the tree canopies are also critical for sugar accumulation and fruit sweetness through more enhanced levels of photosynthesis. The patterns of resource distribution (nutrients, carbohydrates, and water) can significantly be influenced by light. Abundant light availability in fruit producing canopy regions can direct resource partitioning to fruit rather than other parts of the tree. The amount of light and its brightness influences carbon competition and partitioning (source-sink relationship) among tree organs and how it is used for vegetative growth and reproduction. Thus, light has two important effects on the production of high-quality fruit. First, it provides energy that fruit trees store as carbohydrates. Second, it promotes growth and shapes tree structure, improving physical features in areas of the canopy that are well illuminated (Corelli-Grappadelli, 2003). Because of the numerous impacts of light, understanding the fundamental relationships between light and tree physiology is essential for managing the canopy to optimize its production efficiency and to avoid the susceptibility of sunburn (Corelli-Grappadelli, 2003; Musacchi and Serra 2018).

3.2.2. Temperature

Every aspect of apple production is significantly impacted by temperature. First, temperature establishes the limits of the suitable areas for apple production. The cultivated apple, which is essentially a temperate deciduous tree, requires a significant period of winter chill to break dormancy. Thus, regions with warm winters are not suitable for apple production. Second, the duration of growing season is determined by temperature, restricts the number of cultivars that can be cultivated in each area based on their harvest time. Third, temperature affects the rates of all physiological and metabolic functions of the plants, including respiration, cell division, and pollen tube germination and growth. Fourth, the development of plant pathogens and pests is influenced by temperature therefore warmer regions generally exhibit higher disease pressure or a greater number of insect generations compared to cooler places (Palmer et al., 2003). In addition, during bloom time apple flowers are susceptible to cold damage and loss due to exposure to lethal temperatures or to frost ring development due to exposure to non-lethal low temperatures (Fig.1.5) (Palmer et al, 2003). The risk of spring frost damage can be minimized using late-flowering varieties or by choosing areas that have a low chance of spring frosts because of their location, slope, and orientation, and have good cold-air drainage. Frost control systems that can provide a level of protection during bloom time are overhead irrigation, wind machines, and different types of air heaters including propane heaters. On the other hand, high temperatures during the growing season create serious problems in cropping systems including apple production systems (Medda et al., 2022; Do, et al., 2024). Insufficient apple fruit skin red over-color is one of the many negative effects of high temperatures during the last phase of the growing season and close to harvest time (Fig. 1.4). Apple fruit's natural coloring process is disrupted by high temperatures during fruit development by the inhibition of biosynthesis of anthocyanins and carotenoids (Koshita, 2015; Do et al., 2024). In addition to color development limitation, high temperatures increase the susceptibility to sunburn and cause firmness loss by speeding up cell wall dismantling the cortex tissue of the fruit, resulting in early softening and a loss of its crisp texture (Ebel et al.,1993; Lachapelle et al., 2013).

3.3. Orchard management

A complex and balanced interplay between environmental and orchard management factors determines fruit quality development in fruit crops. Growers should be able to increase the percentage of fruit packed in the highest quality grade as well as the average quality of their crop by knowing how the environment, cultural management, harvest and postharvest handling, and storage conditions affect fruit quality for the consumers (Bound., 2005). Hence, the most essential elements to achieve the required product quality, given a certain genetic background, are environmental and management variables throughout the growth season (Wünsche et al., 2010). Before establishing a new orchard site, it is necessary to think about the production system that will be employed. When setting up a fruit orchard, it's important to think about things like soil characteristics and when and how to prepare the ground for planting, water quality and availability as well as the irrigation method and planting densities.

3.3.1. Irrigation and nutrition

Adequate irrigation is essential for tree health and optimum fruit yield and quality. Water stress resulting from waterlogging conditions or insufficient soil moisture may lead to heightened occurrences of corking and bitter pit. Water stress may lead to decreased fruit size and atypical fruit shape (Naor et al., 1999; Wünsche et al., 2000; Musacchi and Serra, 2018). Over-irrigation causes soil-borne diseases, affecting root health and the capacity of roots to absorb minerals like calcium. Excessive irrigation close to harvest can also cause watercore disorder (Fig. 1.3). Opara et al. (1996) revealed that changes in soil moisture, especially occurring late in the season after a period of dry weather, might induce apple skin splitting and moisture loss.

Tree health, growth, productivity and fruit quality is highly correlated with balanced mineral nutrition. Bünemann (1980) reported that fruit quality is frequently impacted by a deficiency or excess of a single element. Bitter pit is one of the most significant disorders associated with mineral nutrition in apples. Bitter pit is linked to low calcium levels and especially to the imbalance between calcium with nitrogen, potassium and magnesium. Over supply of nitrogen or potassium causing high vigor or contitions that favor large fruit size can increase symptom incidence and severity. The symptoms are usually on the lower half of the fruit and in severe cases the bitter pit spotting extends to the upper half. Symptoms usually develop during the first 4-6 weeks of storage, however, in severe situations bitter pit pressure may appear preharvest. Pre-harvest calcium sprays and postharvest immersion to calcium chloride solutions is the most effective treatment for this important nutritional disorder for apples (Bramlage et al., 1980; Musacchi and Serra, 2018).

3.3.2. Fruit canopy position

Apple fruit quality is greatly affected by the position of the fruit within the canopy. As has been mentioned in several research studies (Tustin et al., 1988; Hopkirk et al., 1989; Lawes et al., 1989; Lewallen, 2000; Hagen et al., 2007; Nilsson and Gustavsson, 2007; Jakopic et al., 2009; Drogoudi and Pantelidis, 2011; Hamadziripi, 2012; Kaučić et al., 2023), there is a close connection between the position of the fruit within the canopy, mainly because of the light environment and the microenvironment that occurs in that part of the tree (e.g., sunlight exposure and temperature). Canopy position affects fruit appearance and quality traits, including color (Hamadziripi, 2012; Steyn et al., 2004; Kaučić et al., 2023) and size (Zabedah et al., 2007; Tahir et al., 2007; Kaučić et al., 2023). Moreover, the location of fruit within the canopy may influence the dry matter content (DMC), soluble solids concentration (SSC) (Tustin et al., 1988; Hagen et al., 2007; Hamadziripi, 2012; Léchaudel et al., 2007) and titratable acidity (TA) (Lawes et al., 1989), which are all critical determinants of fruit flavor, significantly impacting apple consumer acceptance (Harker, 2001).

3.3.3. Training systems

Unpruned apple trees grow excessively large and exhibit forking branching, resulting in a dense canopy that obscures the interior of the tree and inhibits light penetration (David and James, 2003). Pruning can be used to improve the shape of trees, control their size, increase floral bud initiation, reduce alternate bearing, enhance fruit quality, and promote light and spray penetration within the tree canopy. On the other hand, training describes the actions that influence tree growth and the evolution of its structural shape. Pruning is basically one of the available training methods. Training is primarily limited to the time when the tree is establishing, though some training can be required once the tree enters production. Improving light distribution is the aim of both training and pruning, ensuring that as much of the tree canopy continues to produce high-quality fruit as possible (David and James, 2003; Ferree and Warrington, 2003). The severity of pruning is influenced by the rootstock, age of the tree, vigor of the tree, and management goals (Andersen, 1984). There are two fundamental categories of pruning cuts, each providing a distinct function: heading cut by cutting the tip of the shoot only or by cutting the tip closely to a lateral branch and thinning cut with which, undesired side branches are completely removed (Andersen, 1984; Appleton et al, 2009).

There are three main systems that have been successfully used to train apple trees: the central leader, the modified central leader, and the multi-leader. The central leader is the standard system for apple tree training which promotes the growth of a taller tree. The cone-shaped tree makes good use of light, but it's hard to set up this training method in windy regions (Andersen, 1984). Modern variations of the central leader are the Tall Spindle Axe (TSA) and the Slender Spindle Axe (SSA) that have been developed for High Density Plantings (HDP). One important characteristic is that the main central leader stays in place of the whole tree's life, instead of being taken out every seven to ten years like in the modified central leader system.

The modified central leader is the most often utilized training system in traditional Low-Density Planting (LDP) systems, creating a tree with four to nine scaffold limbs connected to a robust central trunk. The outcome is a multiple-elder tree, but we begin with a central leader system. The apical dominance manifestation of the temporal central leader promotes the growth of scaffold limbs with broad crotch angles. To grow slightly to one side rather than straight up, the central leader will be guided to a lateral that is between 7 and 10 feet tall. To improve light penetration, the top center will be trimmed down. To make spraying and harvesting easier, tree heights will be maintained below that of the central leader-trained tree. The scaffold limbs should be uniformly distributed around the tree, beginning at a height of around two feet and spaced around eight to twelve inches apart vertically.

Compared to a tree pruned to a central leader, a multi-leader trained tree would be shorter with wider canopy. Wire spreaders are essential for limb spreading to prevent scaffolds from growing vertically. The tree during establishment should be trained with the goal of heading the main leader back and keeping the broader, better-positioned laterals as scaffolds while removing the others so no leader is permitted to take over as the primary leader. With this system, there might be four scaffolds or as many as eight left in the final established tree.

3.3.4. Crop load

Apple fruit quality is influenced by several preharvest factors as mentioned extensively in this chapter. Among all factors that affect fruit quality crop load management is the most influential one as it affects fruit growth, development, maturation and harvest quality (Serra et al., 2016). Crop load is defined as a measure of orchard productivity based on the amount of fruit produced per tree or branch unit (Wünsche et al., 2005; Racskó, 2006). Adjusting the crop load by thinning, a technique used to remove excessive flowers or fruitlets from apple trees, can significantly improve fruit quality through the alteration of fruit-to-leaf ratio. Thinning modifies the availability of carbohydrates (source-sink relationships) for the remaining tree organs, and this affects fruit size, internal and external fruit quality at harvest, return bloom, and the fruit quality during postharvest (Minas et al., 2018). In the rest of the chapters of this dissertation a detailed study focuses on the role of crop load as a significant orchard factor on apple harvest and postharvest quality and that is based on physiological and metabolomics approaches.

4. Conclusion

Apple is a significant fruit crop globally, with the United States being the third-largest producer. Apple has been cultivated for over two millennia, with over 10,000 identified varieties. Its health benefits include phytochemicals like phenolics, flavonoids, and carotenoids that help in mitigating chronic illnesses, malignancies, asthma, and diabetes. The quality of apple fruit is determined by its physical features, mechanical attributes, sensory characteristics, nutritional value, and aesthetic appeal. Understanding the influence of preharvest factors on apple quality and ensuring its preservation through suitable postharvest handling may maintain quality throughout storage and promote increases in apple consumption. Factors influencing harvest quality and postharvest performance include environmental conditions (temperature, light), genotype (cultivars and rootstocks), and orchard management, (irrigation, mineral nutrition, pruning and training, crop load management). Quality is intricately linked to cultivar, with several quality features being inherited. Rootstocks affect tree characteristics, such as vigor limitation and yield efficiency, disease resistance, soil adaptation, and cold hardiness. Sunlight is a critical preharvest component influencing fruit quality and maturity, and understanding the intrinsic interactions between light and tree physiology is crucial for optimum canopy management and the selection of the appropriate training system. Among all orchard management factors crop load is the most influential determinant of the final apple fruit quality. For this reason, this research examines the impact of crop load on apple growth, development and harvest quality, and on postharvest quality using physiological and metabolomic methodologies.

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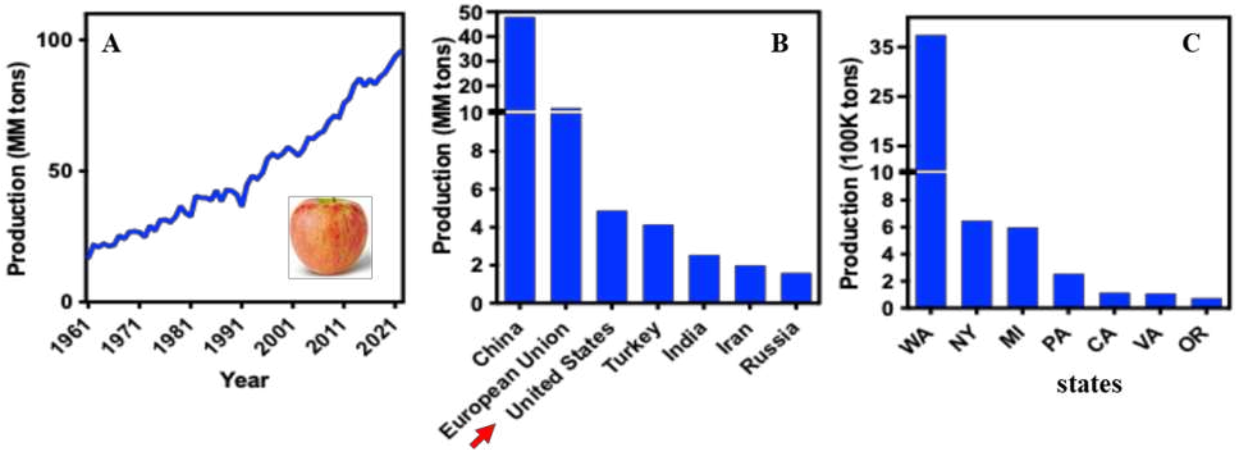


Fig. 1.1 Apple production in the world and US. (A) Apple world production (1966-2022). (B) Major producing countries. (C) Major producing states in US (USDA-NASS, 2024)

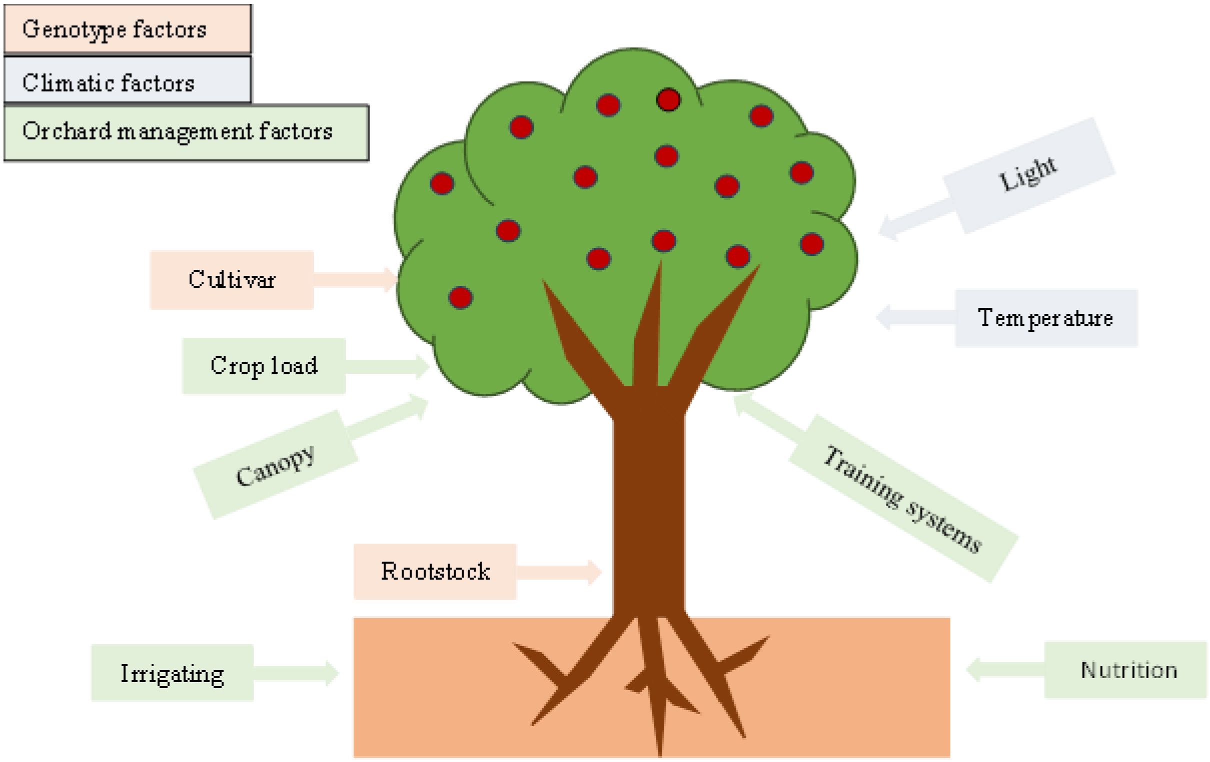
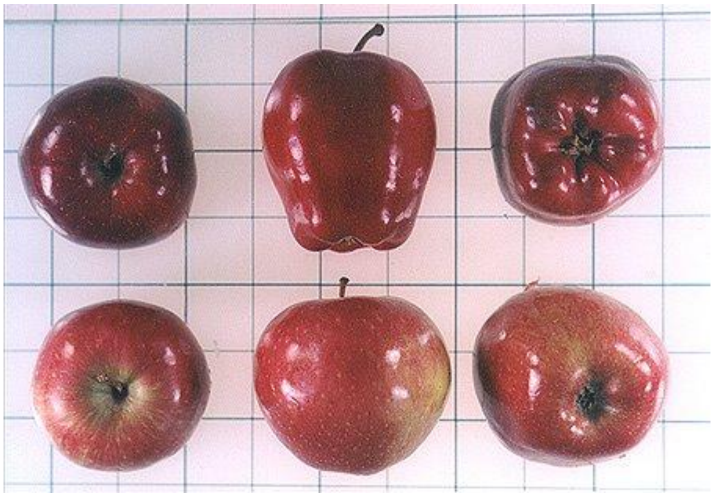


Fig. 1.2. Preharvest factors affecting apple fruit quality consolidated in genotype factors, climatic factors, orchard management factors.

Fig. 1.3. Watercore disorder in apple fruit due to high temperatures and/or over irrigation close to harvest time.



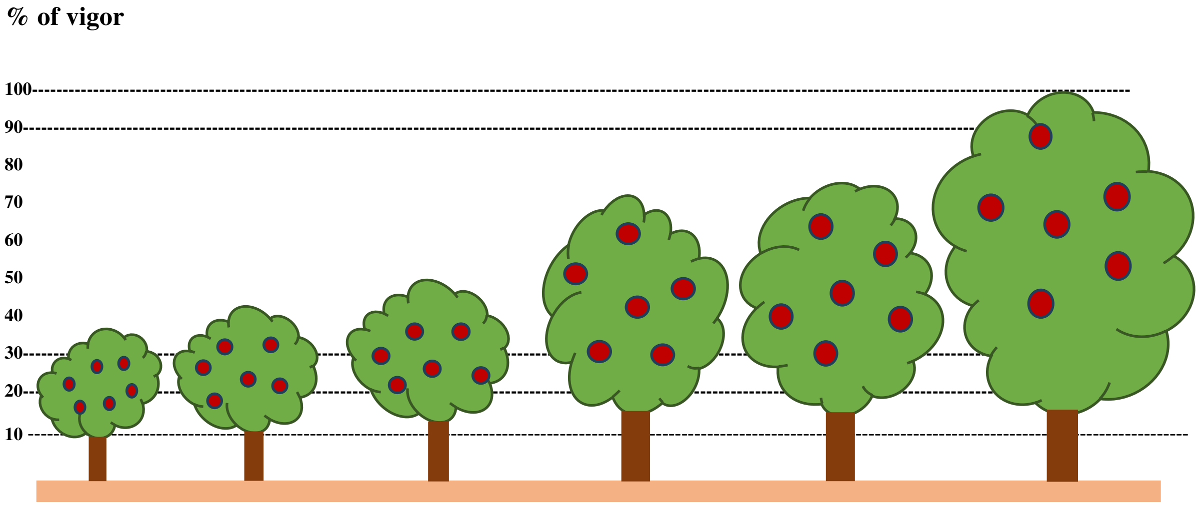
Low temperature

High temperature

Fig. 1.4 High temperatures during the growth season and before harvest reduce apple red over-color blush while low temperature increases the red over-color blush.

Fig. 1.5. Apple frost ring caused during full bloom phase when the flowers exposed to frost damage

Fig. 1.6. Full exposure to sunlight and high temperature cause sunburn in apples



15-30% Size of seedling	**30-35%** Size of seedling	**40-50%** Size of seedling	**40-50%** Size of seedling	**50-75%** Size of seedling	**Over 75%** Size of seedling
Mark	M9 T337	M9 Pajam2	M.26	M.7	B.118
B.9	G.11	G.935	G.30	MM 106	M.13
M.27	G.41	G.222	G.969	MM 111	M.25
P.22	G.16	G.202	G.210	P.2	MAC 24
B.491	G.213	G.214	G.890	P.18	
	B.10	G.814	MAC 46	MAC 1	
			MAC 39	B.490	
			CG.44	M.2	
				M.4	

Fig.1.7. Genotype of apple rootstock and their vigor classification. Vigor categorization is divided as follows: Vigor rootstocks are over 75%, from 40-75% are Semi-dwarf rootstocks and from 15-35% are dwarfing rootstocks.

CHAPTER TWO

INFLUENCE OF CROP LOAD ON APPLE FRUIT GROWTH, DEVELOPMENT, AND HARVEST QUALITY

1. Introduction

Apple (*Malus x domestica* Borkh.) is the fourth most important fruit crop, following citrus, banana, and grape, in the world and in the United States and the second largest fruit crop following peach in Colorado (FAO, 2023; USDA-NAAS, 2022). Current regulation and consumer demand provide high economic value for fruits with superior sensorial and nutritional qualities. Improvement of apple fruit quality is impossible postharvest, as it can only be improved at the preharvest stage (Musacchi et al., 2018). There are many important preharvest factors that impact fruit quality including genotype (e.g., cultivars and rootstock), climate (e.g., temperature), and orchard management (e.g., irrigation, canopy position, and crop load). Among them the most effective preharvest factor to enhance tree fruit quality development is crop load management (Serra et al., 2016; Anthony and Minas, 2022). Crop load management is defined as the number of fruits remaining on trees after flower or fruit thinning (Racsko, 2006). Thinning provides current season benefits for the growers such as increase of fruit size, dry matter content (DMC), soluble solids concentration (SSC) and red over-color coverage, and next season benefits such as decrease of the risk for alternate bearing (Serra et al., 2016; Minas et al., 2018; 2021; Anthony et al., 2020). Numerous reports on many apple cultivars have demonstrated that crop load management reduced fruit yield per tree but at the same time increased fruit size at harvest or caused the reduction of the amount of smaller fruit sizes at harvest (Forshey and Elfving, 1977). The accumulation of photosynthates (sorbitol and sucrose) that are synthesized in leaves by photosynthesis in fruits is enhanced by the reduction of fruit (e.g., sink) number because of the enhancement of sink strength (Yamaki, 2010). The rate of assimilate accumulation into a sink is related to its growth potential and this can be increaced by the reduction of competition for carbohydrates between sinks (DeJong and Grossman, 1995; Becker et al., 2015). Overal, thinning flowers or fruitlets changes the leaf-to-fruit ratio, or the source/sink relationship for carbohydrate distribution. Reducing the potential yield is critical to reduce the competition for the products of photosynthesis between the remaining fruits, so there is an adequate pool of carbohydrates through growth and development enabling them to reach maximum growth and quality potentials at harvest (Grossman and DeJong, 1995; Anthony et al., 2020).

The assessment of the effect of crop load on apple fruit quality and maturity during growth and development can be done destructively and/or non-destructively. Destructive methodologies represent the standard industry practice to assess fruit quality and harvest indices. These types of methods are well established but they are time-consuming, labor-intensive, and can not be used in the field for large numbers of fruit samples in a tree canopy. On the contrary, non-destructive methods are more friendly for large-scale data acquisition in the orchard, with higher frequency, and for multiple parameters even on the same fruit during the growing season (Minas et al., 2018; 2021). A novel calibration protocol for accurate non-destructive measurements of peach fruit quality and maturity was developed by Minas et al. (2021) using visual (Vis) to near infrared spectroscopy (NIRS). Using Vis-NIRS the were able to accurately assess peach fruit quality parameters (DMC and SSC) and physiological maturity based on the index of absorbance difference (I_{AD}) simultaneously with one scan (Minas et al., 2021). I_{AD} is considered a new index that has been developed in recent years to analyze chlorophyll degradation that occures during fruit maturation process (Minas et al., 2018). A similar methodology was followed to develop accurate non-destructive models for rapid apple maturity and quality assessment (Minas et al., unpublished).

The aim of the present study was to determine the effect of crop load management on ‘Gala’ apple quality and maturity during different growth and development stages and at harvest. The hypothesis tested was: crop load related modification of carbohydrates availability (sourse-sink relationship) and fruit-to-fruit competition during fruit growth and development will affect apple fruit quality and maturity at harvest. To test our hypothesis, standard destructive and novel non-destructive techniques were used to assess quality and maturity on apples coming from different crop loads during one growing season.

2. Materials and methods

2.1. Plant material and experimental design

Twelve apples (*Malus domestica* L. Borkh., cultivar ‘Gala’) trees of uniform size and health were selected from Western Colorado Research Center- Orchard Mesa (WCRC-OM), Grand Junction, CO (39°02′31.3″N,108°27′56.8″W) at the Colorado State University’s Experimental Orchard. The trees were 10 years old, grafted on M111 rootstock, and trained to an open center with 5 m in-row and 5 m intra-row spacing and a planting density of 485 trees · ha^{-1}. Standard commercial horticultural care was applied to all trees during the season except for thinning which was adjusted to create four crop load treatments. Full bloom was observed on April

12, 2016. Fifty-four days after full bloom (DAFB), hand thinning was used to achieve the crop load treatments (heavy, commercial, light) on three ‘Gala’ apple trees per treatment, and three trees were left unthinned to be used as a control. The different thinning levels were applied to create various levels of carbohydrate competition between growing apple fruit. The crop load levels created were 1614 fruit/tree for the unthinned trees, 978 fruit/tree for the heavy crop load, 598 fruit/tree for the commercial crop load, and 380 fruit/tree for the light crop load. The trunk cross-sectional area (TCSA) was also measured as previously described (Minas et al., 2023) at 30 cm above the graft union to express the crop load treatments in a form that minimizes the effect of tree size.

To get more detail information about the effect of crop load on fruit growth and development, 30 fruits were flagged from three ‘Gala’ trees per crop load treatment (10 fruits per tree) immediately after thinning and their diameter was recorded using a digital caliper every week for the rest of the growing season until harvest. The apple fruit was considered as a sphere and fruit diameter data used to calculate volume. At three different developmental stages (77, 99, and 129 DAFB) 10 fruit per tree (30 fruit per crop load treatment were sampled to assess fruit size (mm), overcolor blush coverage (%) and internal fruit quality (DMC and SSC) and maturity parameters (flesh firmness, FF and I_{AD}).

2.2. Fruit quality and maturity analysis

Fruits were harvested at commercial maturation on August 19, 2016 (129 DAFB). The number of fruits per tree was counted and then weighed to determine yield per tree (kg. tree $^{-1}$). Fruit count per cm^2 of TCSA (fruit no. · cm^{-2} of TCSA) was calculated to determine the crop load. One hundred fruits per tree were randomly sampled from the 3 experimental trees per treatment to determine the effect of crop load on fruit size, fruit red overcolor blush, physiological maturity and internal quality attributes. The fruit size was measured with a produce measuring gauge (Cranston Machinery Co, Inc., Oak Grove, Oregon) and expressed as the perimeter (mm) and the fresh weight (FW) expressed as (g) was measured of each fruit to study the effect of crop load on fruit size using a digital scale (model COURIER 3000 I-C31M75L, OHAUS CORPORATION, Parsippany, NJ). Fruit red overcolor blush coverage was rated subjectively using percent coverage method and expressed as percentage (%). To assess the effect of crop load on apple fruit internal quality and maturity at harvest we used both destructive and non-destructive methods on these one hundred fruit per tree and then we compared the results of the two methods to validate the performance of the non-destructive methods. Destructively, dry matter content (DMC) was measured by taking the fresh weight (FW) of apple cortex samples after peeling and removing a 25-mm core using a digital scale (TC-204, Denver instruments, Arvada, CO, USA), and then putting the samples in a forced air oven (VWR Oven F Air 104 L, VWR, Radnor, PA) at 65°C for 3 days to dehydrate and then the dry weight (DW) was recorded to calculate DMC (%). Soluble solids concentration was assessed destructively by using a digital temperature compensated refractometer (PR-32α, Atago, Tokyo, Japan) by taking a 25-mm core sample from the apple cortex and juiced using a handheld garlic press and then put the juice drops onto the prism of the refractometer to record the percentage (%) of SSC.

Fruit maturity was assessed with three parameters: the flesh firmness (destructive) and the index of absorbance difference (I_{AD}, non-destructive) and the starch index (SI, destructive). Flesh firmness (FF) was determined with a digital fruit texture analyzer (model FR-5120, Lutron Electronic Enterprise Co., Taipei, Taiwan) equipped with a 12-mm diameter stainless probe.

Analysis was accomplished on a single side per fruit that was previously peeled with 1-mm thick skin tissue removed. Individual fruits were analyzed on opposite sides at the equatorial region (Minas et al., 2021). Results that corresponded to the maximum force applied by the probe to penetrate into the fruit flesh by 10 mm were expressed in newtons (N). The physiological maturity was assessed with the index of absorbance difference (I_{AD}) by using visible to near infrared spectroscopy (Vis-NIRS) through a 'closed type' handheld sensor that is factory calibrated (DA-meter®, T.R. Turoni srl, Forlì, Italy) (Costa et al., 2009). This index measures the difference between two light wavelengths (I_{AD} = A670nm-A720nm) that the chlorophyll absorbs through the fruit surface reflecting the background color that is correlated to the fruit physiological maturity (Anthony and Minas. 2022). Starch index (SI) was measured by using a starch-iodine test which is the standard for the apple industry to define commercial harvest maturity. The starch index (SI) was assessed at the end of the process using a 1-cm thick slice from the equatorial region of the fruit and sprayed with Lugol's solution (15 $g \cdot L^{-1}$ potassium iodide and 6 $g \cdot L^{-1}$ iodine) using a handheld spray bottle. By applying Lugol's iodine to the fruit slides two different areas showed a clear area in the fruit slide representing the regions where the starch had already converted into sugar and the dark blue areas representing the regions of the high starch value in the fruit slide. This method is particularly valuable for fruits like apples and pears, where the degree of starch degradation correlates with sugar accumulation, flavor development, and post-harvest performance. Assessments were made after at least 5 min incubation and no later than 30 min after application. SI was visually rated on a 1 to 9 scale according to the Michigan State University Scale (1 = no hydrolysis, all tissue stained black; 9 = hydrolysis complete, tissue white; Beaudry, 2023) (Figure 2.1).

In addition to the destructive assessments, an ‘open type’ non-destructive handheld sensor was used for rapid internal quality and maturity assessments. This Vis-NIRS sensor (F-750 Produce Quality Meter, Felix instruments Inc., Camas, WA, USA) has been previously calibrated as by Minas Lab to non-destructively estimate peach (Minas et al., 2021; 2023) and apple (Minas et al., unpublished) fruit internal quality based on dry matter content (DMC, %) and soluble solid concentration (SSC, %) at 729 to 935nm and physiological maturity (I_{AD}) at 600 to 740 nm.

2.3. Non-targeted metabolite profiling using gas chromatography mass spectrometry (GC-MS)

Metabolite extraction was conducted by weighing 25 mg of each freeze-dried apple cortex sample and placing them into clean 2 mL autosampler glass vials (VWR, Radnor, PA, USA). One mL of 80% by volume LC-MS grade methanol (MeOH) in water solution was then added to each vial and vortexed on a plate shaker (Fisherbrand^{TM} Analog Multitube Vortexer, ThermoFisher Scientific, Waltham, MA, USA) at 4 ^{o}C at max speed (2500 rpm) for 2 hours (h). Samples were then held at -80 ^{o}C for 1 h followed by centrifugation for 25 min at 3500 rpm at 4 ^{o}C. The supernatant was extracted (~800 µL) without disturbing the pellet and pipetted into new a 2 mL autosampler vial. A pooled quality control (QC) solution was created by transferring 10 µL of each sample into a separate glass vial. Fifteen µL of each sample was transferred to another set of glass vials, centrifuged for 2 min at 3500 rpm and then dried under N_2 (g) for 30 min. Dried samples were stored at -80 ^{o}C until derivatization. Derivatization (methoximation and silylation) took place immediately prior to running the samples. Dried down samples were allowed to warm to room temperature and then re-suspended in 50 µL of methoxyamine HCl (prewarmed to 60 ^{o}C) and centrifuged for 30 sec. Samples were then incubated at 60 ^{o}C for 45 min, followed by a brief vortex, sonication for 10 min and an additional incubation at 60 ^{o}C for 45 min. Following this, the samples were centrifuged before receiving 50 µL of N-Methyl-M (trimethylsilyl) trifluoroacetamide (MSTFA) + 1% trimethylchlorosilane (TMCS) (ThermoFisher Scientific, Waltham, MA, USA), briefly vortexed and incubated at 60 °C for 40 min, as described previously (Chaparro et al., 2018). Samples were loaded (~ 80 µL) into glass inserts within glass autosampler vials and centrifuged for 30 sec prior to GC-MS analysis.

GC-MS analysis was performed using the Clarus 690 GC coupled to a Clarus SQ 8S Mass Spectrometer (PerkinElmer, Waltham, MA, USA). Metabolites were separated with a 30 m TG-5MS column (Thermo Scientific, 0.25 mm i.d. 0.25 µm film thickness). The GC program began at 80 °C for 0.5 min and was ramped to 330 °C at a rate of 15 °C per minute and ended with an 8 min hold at a 1 mL $\cdot$ min^{-1} helium gas flow rate. The inlet temperature was held at 285 °C and the transfer line was held at 260 °C. Masses between 50-620 m/z were scanned at four scans/sec after electron impact ionization. QC injections were analyzed after every 6th sample and were used to control and detect analytical variation.

Metabolomic data processing was conducted as previously described (Chaparro et al., 2018). GC-MS files were converted to .cdf format and processed by XCMS in R (Smith et al., 2006; R Core Team, 2015; Mahieu et al., 2016). All samples were normalized to the total ion current (TIC). RAMClustR was used to deconvolute peaks into spectral clusters for metabolite annotation (Broeckling et al., 2014). RAMSearch (Broeckling et al., 2016) was used to match metabolites using retention time, retention index and matching mass spectra data with external databases including Golm Metabolome Database (Hummel et al., 2007; Hummel et al., 2013) and NIST (Broeckling et al., 2016).

2.4. Statistical analysis

JMP Pro13 software (JMP® 13 Automation Reference Copyright © 2016, SAS Institute Inc., Cary, NC, USA) has been used to assess the statistical differences of all quality and maturity parameters between thinning treatment during growth, development and at harvest. Tukey (HSD) was used to assess the difference between mean values of fruit yield, size, SI, DMC, SSC, FF, and I_{AD} at (P<0.05). Principal component analysis (PCA) was conducted on metabolomic data utilizing SIMCA (Umetrics, Umea, Sweden).

3. Results and Discussion

3.1 Crop load impact on fruit quality and maturity during apple fruit growth and development

Among the various orchard and preharvest factors that affect temperate tree fruit appearance, internal quality, and maturity, crop load management by thinning is the most impactful (Minas et al., 2021). To test the effect of crop load on 'Gala' apple fruit quality (fruit volume, color, size, weight, DMC, SSC) and maturity (FF, I_{AD}) during growth and development, trees at different crop load treatments were sampled at different developmental stages (77, 99 and 129 DAFB). Crop load significantly affected apple fruit volume (cm^3) during growth and development and at harvest. The effect of thinning on fruit volume started to be observed after the period between 77 and 99 DAFB, (Figure.2.2). There was a 10.3% increase in fruit volume between light crop load and commercial crop load apples at 99 DAFB compared to unthinned ones and there were no significant differences in fruit volume between the rest of the treatments at that time point. Light crop load treatment and commercial crop load treatments significantly increased the volume of the fruit by 15.4% at harvest (129 DAFB), compared to unthinned trees (Tucky's HSD test at *P=0.05)*. In addition, the heavy crop load treatment exhibited a 5.6% increase in fruit volume compared to unthinned trees at the final sampling stage at harvest. These results, similar to other studies on apple and peach fruit, have demonstrated that the reduction of crop load improves fruit volume during growth and development (Serra et al., 2016; Anthony et al., 2020).

Results indicated fruit red overcolor improved by more thinning. In (Figure 2.3) it is illustrated that at the early developmental stage (77 DAFB) there was not any difference in fruit color appearance. The first indications of visual differences on fruit appearance started in the commercial and light crop loads at 99 DAFB and a clear improvement on red overcolor blush was visible at harvest (129 DAFB). Anthony et al. (2020) reported similar results in a peach crop load study where the effect of crop load on fruit overcolor was drastically different at the final stage of growth and development. The improvement on color coverage of apple fruit during growth and development is associated with higher light penetration levels through the tree canopy. In this study light penetration was not assessed, however, the trees were similar in size and the only difference was on crop loads. Lighter crop load trees enhanced fruit red coloration mainly because of the availability of carbohydrates early in fruit development that may lead to increased levels of flavonoid synthesis such as anthocyanins as previously reported (Anthony et al., 2023). Fruit fresh wight (FW, g) and fruit size also increased by more thinning during fruit growth and development (Figures 2.4.A and B). There were no significant differences in FW and fruit size between the treatments at 77 DAFB. However, it was found that significant differences in FW and fruit size between the treatments appeared after 99 DAFB, especially between light crop load and unthinned trees by 32.8% for FW and by 11% for fruit size. Similar differences in FW and fruit size were observed at harvest (129 DAFB) between the same treatments. The increase in fruit FW and size with reduced crop load is attributed to increased leaf to fruit ratios that potentially modifies the ability of the fruits (sink) to assimilate more carbon by photosynthesis (DeJong and Grossman, 1995). The effect of crop load on 'Gala' apple fruit internal quality (DMC, SSC) and maturity (FF, I_{AD}) across developmental stages was significant between the unthinned and thinned treatments (Figures. 2.4. C, D, E, and F). In terms of fruit internal quality parameters (DMC, SSC) there were significant positive differences realized early in development (77 DAFB) between the unthinned trees and all other thinning treatments (heavy, commercial, and light crop loads) through all the development stages studied. DMC increased by 9% at 77 DAFB and 7.5% at 99 DAFB in light and commercial crop load treatments compared to untinned trees, meaning that balancing crop load helps maintain increased DMC levels in apple during different developmental stages. SSC also increased as crop load decreased during 'Gala' apple growth and development. The percentage of increase in SSC was 5% at 77 DAFB and 9% at 99 DAFB in light and commercial crop load treatments compared to untinned trees. The increase in SSC during growth development is attributed to starch conversion into sugars over time via hydrolysis (Visser et al., 1968). On the other hand, apple fruit physiological maturation speed ($I_{AD,}$ FF) increased by more thinning during growth and development (Figs. 2.4 E and F). Flesh firmness (FF) decreased throughout growth and development, but there was not a significant difference observed across the four crop load treatments (Fig. 2.4 E). Index of absorbance difference (I_{AD}) advanced with decreased crop load (Fig.2.4F). Low I_{AD} value (Chlorophyll contact) was observed and statistically significant with lighter crop loads at 129 DAFB. Overall, in this study our findings demonstrated that decreased crop loads resulted in enhanced apple fruit appearance, increased fruit internal quality parameters, and advanced fruit maturity during growth and developmental stages.

3.2 The impact of crop load on fruit quality and maturity at harvest

The thinning treatments that were used (heavy, commercial, and light crop loads) had a significant effect on the harvested fruit numbers per tree (no./tree) compared to the unthinned trees (Table 2.1). Fruit no. per square cm of TCSA was used as an index of the actual crop load as it includes the effect of tree size or the capacity of trees to produce fruits because of the size of their canopy. In our experiment there was no significant difference in trees size between the treatments, however, crop load expressed as fruit no. · cm^{-2} of TCSA was significantly different among treatments (Table 2.1). The thinned trees with the highest crop load (heavy crop load) had an average of 978 fruits per tree, the ones with the commercial crop load that has been used as a standard practice by the growers had 598 fruit per tree, and the extreme thinning treatment that gave a light crop load had 380 fruit per tree that were compared to the unthinned trees that had 1,614 fruit per tree. In more detail, fruit hand thinning to heavy, commercial, and light crop load had a significant impact on fruit yield (kg · $tree^{-1}$) at harvest by 30.5%, 52.5%, and 66.9%, respectively, compared to the unthinned treatment. Fruit size and fruit fresh weight increased at harvest with increasing the thinning intensity on 'Gala' apple trees. In terms of fruit size, the percentage of fruits whose size was from 70 mm to >80 mm was 17% for the unthinned trees, 25% for the heavy crop load, 56% for the commercial crop load, and 60% for the light crop load (Figure 2.5). The average fruit size (fruit diameter in mm) also increased by more thinning with 7% and 6% increase in light and commercial crop loads compared to unthinned trees, respectively. The average fruit FW of light crop load treatment at harvest was 157 g which is a 38% increase compared to unthinned trees. In addition, commercial crop load fruit was 143.8 g with a 26.6% increase compared to untinned trees. On the other hand, heavy crop load fruit showed a 13.5% increase compared to unthinned ones in FW at harvest (Table 2.1). Fruits from light crop load trees had significantly redder overcolor blush coverage by 62% more than unthinned trees at harvest (Figure 2.6, Table 2.1). Additionally, light crop load fruit exhibited a 9.7% increase in red overcolor blush coverage compared to commercial crop load. The percentage of fruits whose overcolor coverage was >80% was 19.5% for unthinned, 42.0% for the heavy crop load, 44.6% for the commercial crop load, and 61.5% for the light crop load (Figure 2.6). Here, it is important to mention that overcolor coverage is a perceived marketplace quality attribute for bicolored apples such as ‘Gala’ to get high premium prices and consumer acceptance. A minimum of 70-80 % of coloration is required for high premium prices (Watkins et al., 2002; Dobrzanski et al., 2017). These findings agree with previous reports in ‘Jonagold’ apples that established the overcolor blush coverage is increased at harvest with decreased crop loads (Stopar et al., 2002).

Management of crop load during postharvest significantly enhanced apple fruit internal quality and speeded up the maturation process at harvest. By using both destructive and non-destructive methods to analyze the effect of crop load on fruit internal quality and maturity parameters significant differences in fruit quality (DMC, SSC) and physiological maturity (I_{AD}) were observed in this study across all thinning treatments compared to the untinned trees. Interestingly, the starch index (SI), which represents the standard maturity index for the apple industry when it comes to harvest decisions, showed no significant difference at harvest across all the treatments based on Tueckey’s HSD test (*P*=0.05) (Table 2.2). The average SI values were 19.4% and 47.2% higher for the light and commercial crop loads, respectively, compared to the unthinned treatment, however these differences were not statistically significant given the variability of the samples tested for this parameter. In a previous study, ‘Honeycrisp’ it was reported that the lowest crop load showed the higher SI values at harvest (Serra, et al., 2016). The SI results of the present study show a similar trend to previous reports but also highlights the increased risk for error using this methodology to predict the ideal harvest date of apple fruit.

Dry matter content (DMC) was significantly increased with the reduction of yield and crop load. More specifically, the reduced crop loads gave fruits with increased DMC by 17.6, 14.7, and 9.2% for light, commercial, and heavy crop loads, respectively, compared to the unthinned trees (Table 2.2). DMC is becoming an important index of fruit internal quality and consumer acceptance because it is related to the total amount of carbohydrates indicating the percentage of assimilated carbon by fruit (Palmer et al., 2010). Studies on different apple cultivars have reported that DMC increases with lower crop loads (Anthony et al., 2019; Serra et al., 2016; Palmer et al., 2010), similarly to what has been reported in previous peach studies (Minas et al., 2021; Anthony et al., 2020). SSC also increased with lower crop loads. Our results showed that the light crop load treatment increased SSC by 23.7% compared to unthinned trees at harvest, similarly to what has been previously reported for apple (Serra et al., 2016).

Using the pre-calibrated Vis-NIRS handheld sensor it was possible to assess 300 fruit per crop load treatment (100 fruit per tree) non-destructively for internal quality (DMC and SSC) and physiological maturity (I_{AD}). The non-destructive models had previously been developed by Minas Lab (Minas et al., unpublished) and used in this study to further validate their capacity for rapid apple fruit quality assessment. The readings from the non-destructive sensor were compared with the reference values through regression analysis to characterize the linearity (R^2) and the Root mean squared error of prediction (RMSEP) of the created models for DMC, SSC and I_{AD}. Results of the regression analysis of the validation showed that all three parameters can be estimated with low error using the Vis-NIRS non-destructive sensor by a single scan (Figure 2.7 A, B, and C). More specifically, DMC was measured non-destructively with R^2=0.89 and RMSEP=0.33, SSC with R^2=0.87 and RMSEP=0.47 and IAD with R^2=0.96 and RMSEP=0.05. Similar high accuracy results have been reported previously by Minas et al. (2021; 2023) on peach. The analysis of the large-scale sample sets (n=300) per crop load treatment showed that more thinning increased the percentage of fruit in the high DMC, SSC clusters, meaning that the percent of fruit with >16% DMC was 47.4% in light crop load, 26.7% in commercial crop load, 13.5% in the heavy crop load and only 1.7% in the unthinned treatment (Figure 2.7, D). Similarly, the percentage of fruit with >14% SSC was 91.6% in light crop load, 76.8% in commercial crop load, 22.0% in the heavy crop load and only 1.7% in unthinned treatment (Figure 2.7, E). It is important to mention that consumers can sense just 1% difference in SSC among different fruit samples (Harker et al., 2002).

On the other hand, crop load influenced in apple fruit physiological maturity (I_{AD}) at harvest (Serra et al., 2016). In the present study, FF analyzed destructively, and results showed that there is no significant difference on FF between the treatments (Table 2.2). However, the index of absorbance difference (I_{AD}) as measured accurately non-destructively by using the handheld Vis-NIRS sensor exhibited decreased values at harvest with more intense thinning (Table 2.2, Figure. 2.7 C and F). Fruit from light crop load had 67.5% lowest value of I_{AD} compared with unthinned trees indicating that carbon availability enhances the maturation process similarly to what has been previous reported with other fruit crops like peach (Minas et al., 2021; Anthony et al., 2020). In addition, the results of the present study demonstrate the value of I_{AD} as an important maturity index as it better characterized the effect of crop load in fruit maturation and that future research should focus on this parameter to further implement grower adoption for more precise harvest time decision making.

3.3. Vast metabolic differences shift early on during fruit growth and development as a response to carbon sufficiency/starvation

In total, 169 metabolites were detected in apple mesocarp samples. Of those, 36 metabolites were confidently annotated. Principal component analysis revealed that a large percentage of the metabolic variation is attributed to PC1 (35.5%) representing fruit developmental stage, with additional variation explained by PC2 (28.6%) representing carbon supply manipulation by crop load (Figure 2.8.). Importantly, the largest metabolic separation was observed between the thinned treatment (red symbols) and unthinned control (black symbols) at 77 DAFB (triangles). This separation was increased at 99 DAFB (squares) and significantly reduced at harvest (circles, 129 DAFB). These early shifts in metabolome resulting from variable carbon supply levels due to thinning treatment are in direct contrast to the observed trends in fruit phenotype, which were greatest at the last developmental stage (Figures 2.3 and 2.8). In other words, this early dramatic shift in metabolome (Figure 2.8) is in direct contrast to phenotypic shifts, where differentiation at 77 DAFB in Figure 2.3 is minimal. Overall, shifts in metabolome (Figure 2.8) appear to behave inversely with shifts in fruit quality and maturity attributes (Figure 2.3) and agree with previous reports on peach where it was hypothesized that excess carbon supply early in development it is likely priming a better phenotype at harvest due to the induction of the phenylpropanoid pathway (Anthony et al., 2020; 2023).

4. Conclusion

In conclusion, our results demonstrated that crop load is an important preharvest factor that affect 'Gala' fruit appearance, fruit internal quality, and maturity during growth, development and at harvest. Decreasing crop load by hand thinning reduced 'Gala' apple trees' yield but at the same time increased the percentage of large fruits and fruit fresh weight at harvest. Also, crop load improved fruit red overcolor blush, increased DMC and SSC that used as fruit internal quality parameters during growth, development, and at harvest, also advanced I_{AD} as an important maturity parameter at harvest but did not affect starch index and fruit firmness. Early metabolic shifts during apple growth and development under variable carbon competition treatments indicate that carbon availability or starvation is affecting fruit metabolism and might prime a better phenotype at harvest.

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http://www.nass.usda.gov/

Table 2.1. Effect of crop load on 'Gala' apple yield, fruit size and fruit color at harvest. 'Gala' apple trees grown in Western Colorado were thinned at different levels (heavy, commercial, and light) and compared to the unthinned ones. The effect of crop load on yield is expressed as fruit number per tree or per cm^2 of TCSA and total fruit weight per tree (kg/tree). The effect of crop load on fruit size was expressed as the fresh weight (g) and the perimeter (mm) of each fruit. The fresh weight as well as the size of fruits increased as the number of fruits/tree and yield decreased

Crop load	Crop load (Fruit no. tree^{-1})	Crop load (Fruit no. cm^{-2} of TCSA)	Yield (Kg. tree^{-1})	Fruit FW (g)	Fruit size (diameter, mm)	Overcolor coverage (%)
Unthinned	1613.7 ± 169.7a	0.42 ± 0.03a	181.1 ± 15.7a	113.5 ± 9.7c	65.7 ± 0.3c	45.2 ± 1.7c
Heavy	978.0 ± 39.9b	0.24 ± 0.02b	125.7 ± 2.5b	128.8 ± 3.3bc	67.4 ± 0.3b	63.2 ± 1.6b
Commercial	597.7 ± 29.9bc	0.15 ± 0.01c	86.1 ± 6.2bc	143.8 ± 3.5ab	69.6 ± 0.2a	66.7 ± 1.3b
Light	380.3 ± 26.8c	0.10 ± 0.01c	60.0 ± 6.1c	157.1 ± 5.7a	70.3 ± 0.2a	73.2 ± 1.3a

Mean values ± S.E. followed by the same letter are not statistically different according to Tuckey's HSD test (*P=0.05*).

Table 2.2. Effect of crop load on 'Gala' apple fruit internal quality and maturity parameters at harvest (129 DAFB). Fruits coming from unthinned or thinned 'Gala' apple trees to three crop loads (heavy, commercial, and light crop load) were assessed for internal quality, measured as dry matter content (DMC) and soluble solids concentration (SSC) and maturity, measured as starch index, flesh firmness (FF) and index of absorbance difference (I_{AD}).

Crop load	Starch index	DMC (%)	SSC (%)	Firmness (N)	I_{AD}
Unthinned	3.6 ± 0.6a*	14.2 ± 0.3c	11.8 ± 0.2c	95.8 ± 2.7a*	0.80 ± 0.03a
Heavy	4.6 ± 0.4a	15.5 ± 0.3b	13.3 ± 0.2b	103.8 ± 2.7a	0.48 ± 0.03b
Commercial	5.3 ± 0.7a	16.3 ± 0.3ab	14.1 ± 0.2ab	97.7 ± 3.0a	0.36 ± 0.03c
Light	4.5 ± 0.8a	16.7 ± 0.3a	14.6 ± 0.2a	99.1 ± 2.6 a	0.26 ± 0.03c

*Mean values ± S.E. followed by the same letter are not statistically significant according to Tuckey's HSD test (*P*=0.05)

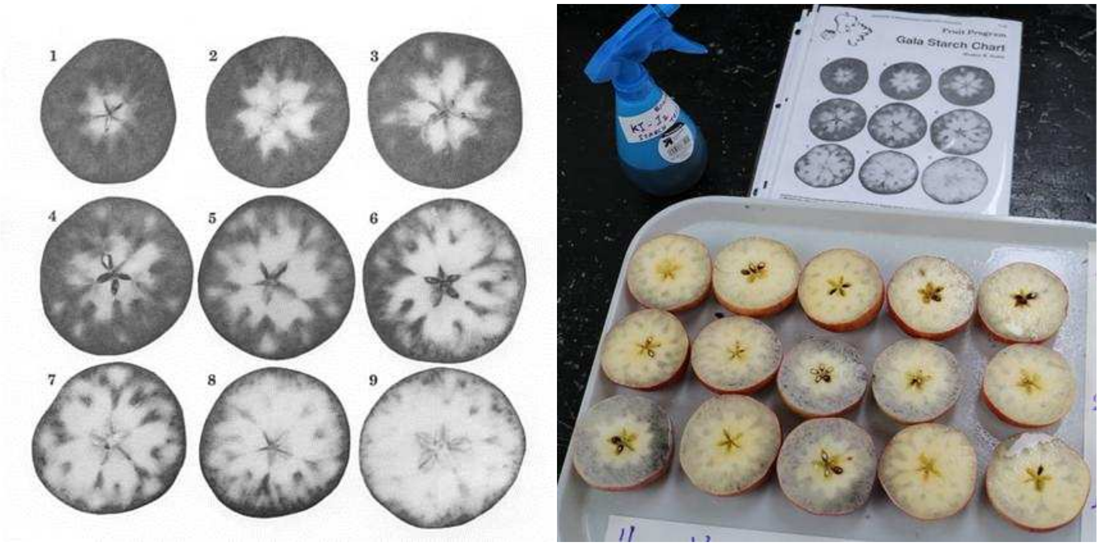

Figure 2.1. Starch index test. Starch-Iodine Test (industry standard) as a commercial harvest indicator when less than 50% of surface is dark purple/black by KI-I_2 solution.

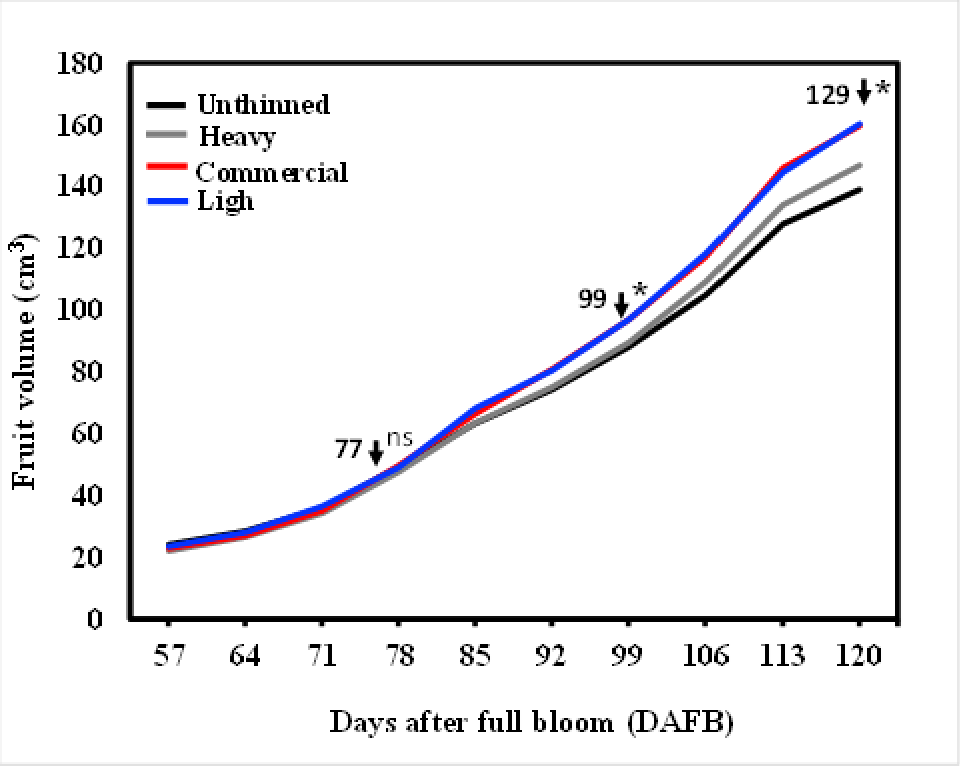

Figure 2.2 Effect of crop load on fruit volume during growth and development for 'Gala' apples grown in western Colorado. 'Gala' apple trees remained unthinned or thinned to three crop load levels (heavy, commercial, and light crop load) at 56 DAFB by hand thinning. Fruit volume was measured in 10 fruit per tree on three trees per crop load treatment every week until harvest (129 DAFB). Arrows indicate the developmental stages sampled for internal quality and maturity analysis. Asterisks (*) indicate statistically significant differences according to Tuckey's HSD test (*P*=0.05) and ns indicates no significant differences for the corresponding time point.

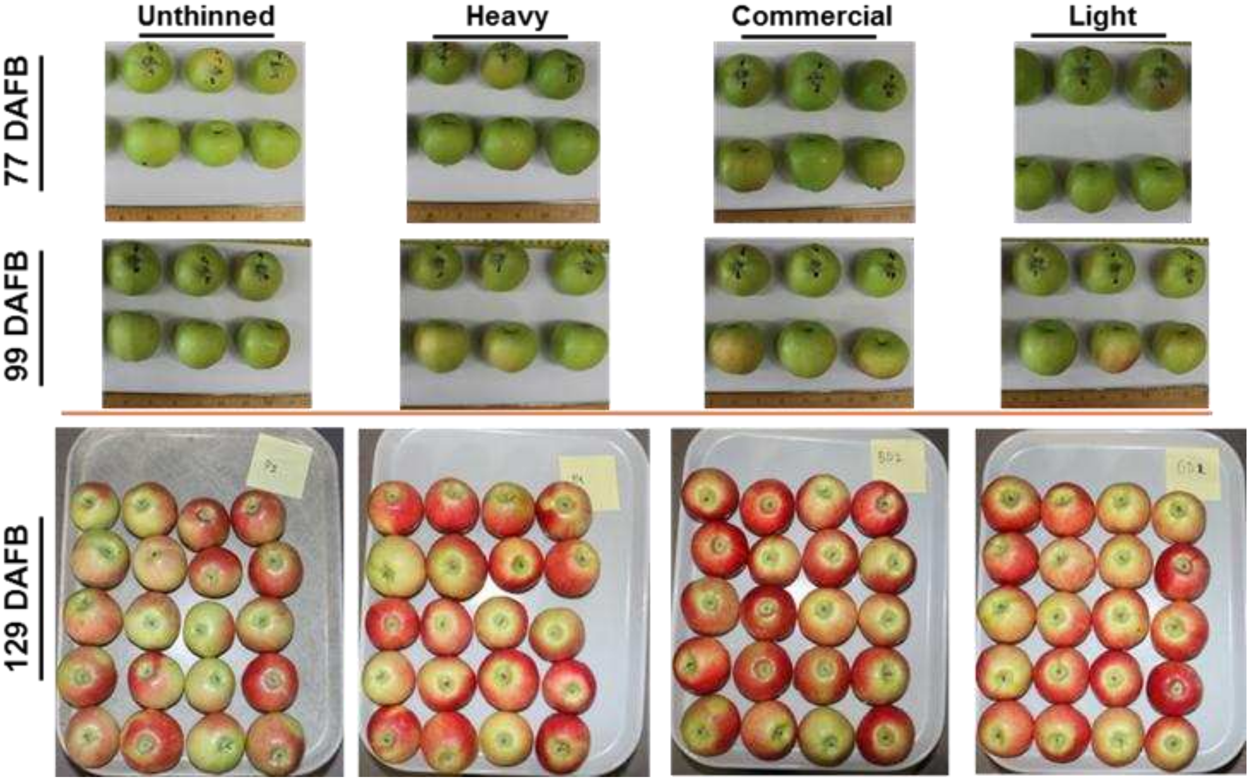

Figure 2.3. Effect of crop load on 'Gala' fruit appearance during growth, development and at harvest. Unthinned and thinned Gala' apple trees to three crop loads (heavy, commercial, and light crop load) were sampled at three different developmental stages (77,99,129 DAFB) to be analyzed for internal fruit quality and maturity.

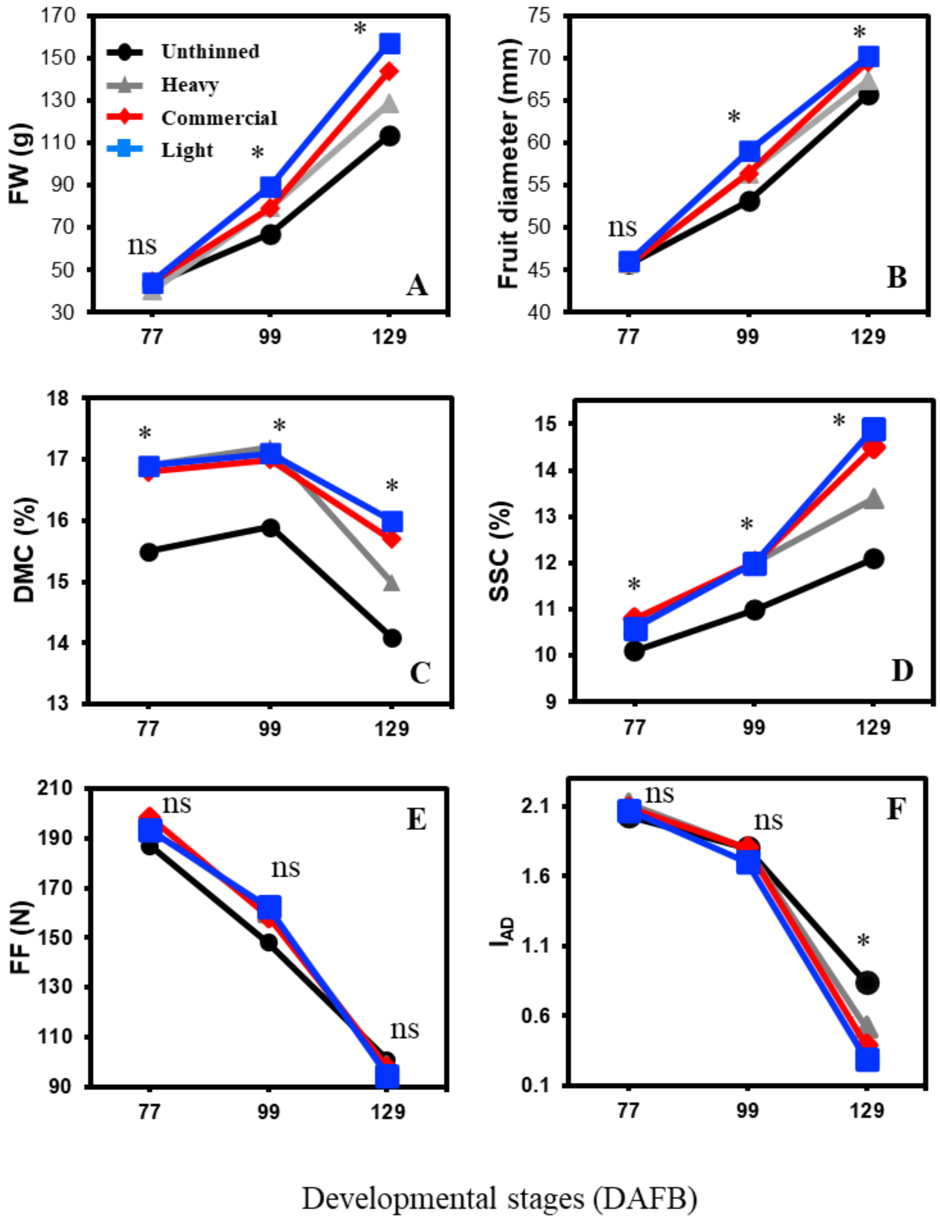

Figure 2.4. Effect of crop load on ‘Gala’ apple fruit quality across developmental stages. Impact of thinning fruit fresh weight (FW, g; A), fruit size (mm; B), fruit internal quality measured as dry matter content (DMC, %; C), soluble solids concentration (SSC, %; D), and on fruit maturity measured as flesh firmness (FF, N; E), index of absorbance difference (IAD; F). Unthinned and thinned ‘Gala’ apple trees to three crop loads (heavy, commercial, and light crop load) were sampled at three different stages (77, 99, and 129 DAFB) during fruit growth, development, and at harvest. The fruit weight and fruit size increased with increase growth and development. Thinning increased fruit internal quality as measured by DMC and SSC at all

tested developmental stages. Fruit maturity was promoted by thinning only in terms of I_{AD} and at harvest stage. Asterisks (*) indicate statistically significant differences according to Tuckey's HSD test (P=0.05) and ns indicates no significant differences for the corresponding time points.

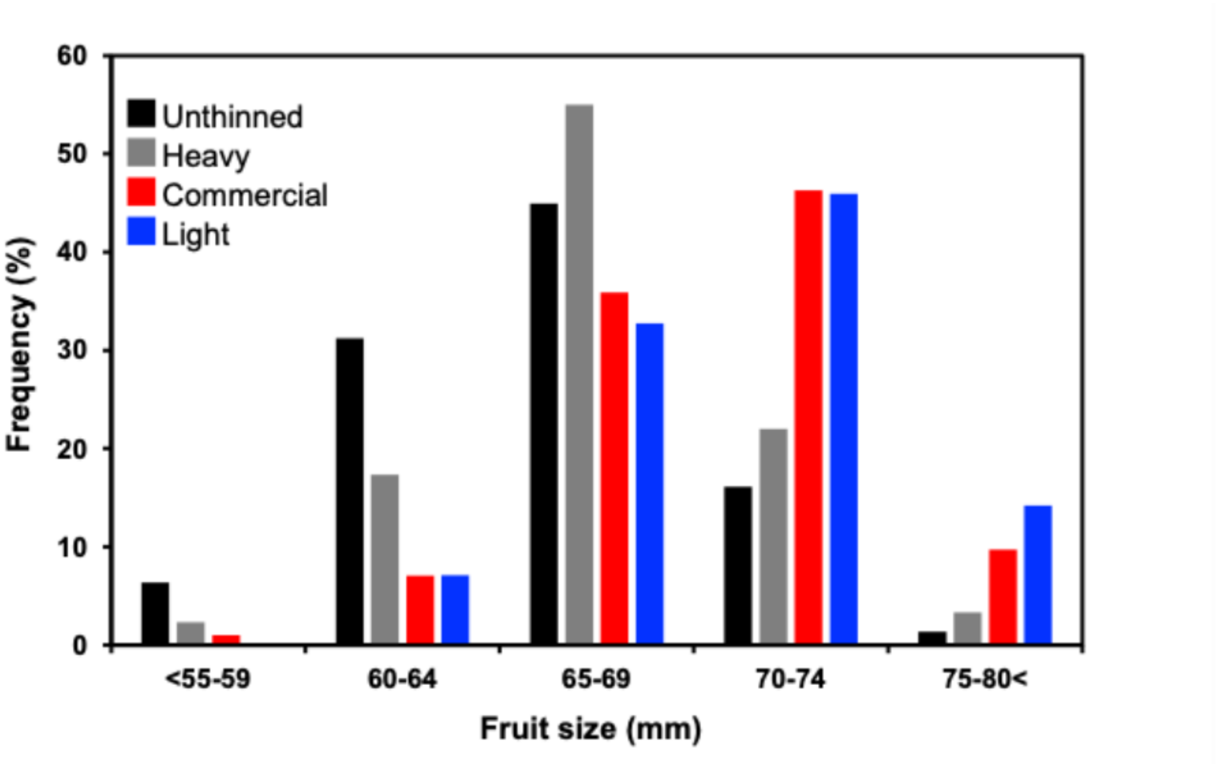

Figure 2.5. Effect of crop load on 'Gala' fruit size (mm) distribution at harvest. Fruit coming from 'Gala' apple trees that were unthinned or thinned to three different crop loads (heavy, commercial, and light crop loads) were measured for their perimeter at harvest. The effect of crop load on fruit size distribution was determined in 100 fruit per tree and 3 trees per treatment and is presented as the percentage of fruits that are classified within different clusters of fruit perimeter. The percentage of apple fruit that were classified in the clusters of larger fruit was increased by more thinning.

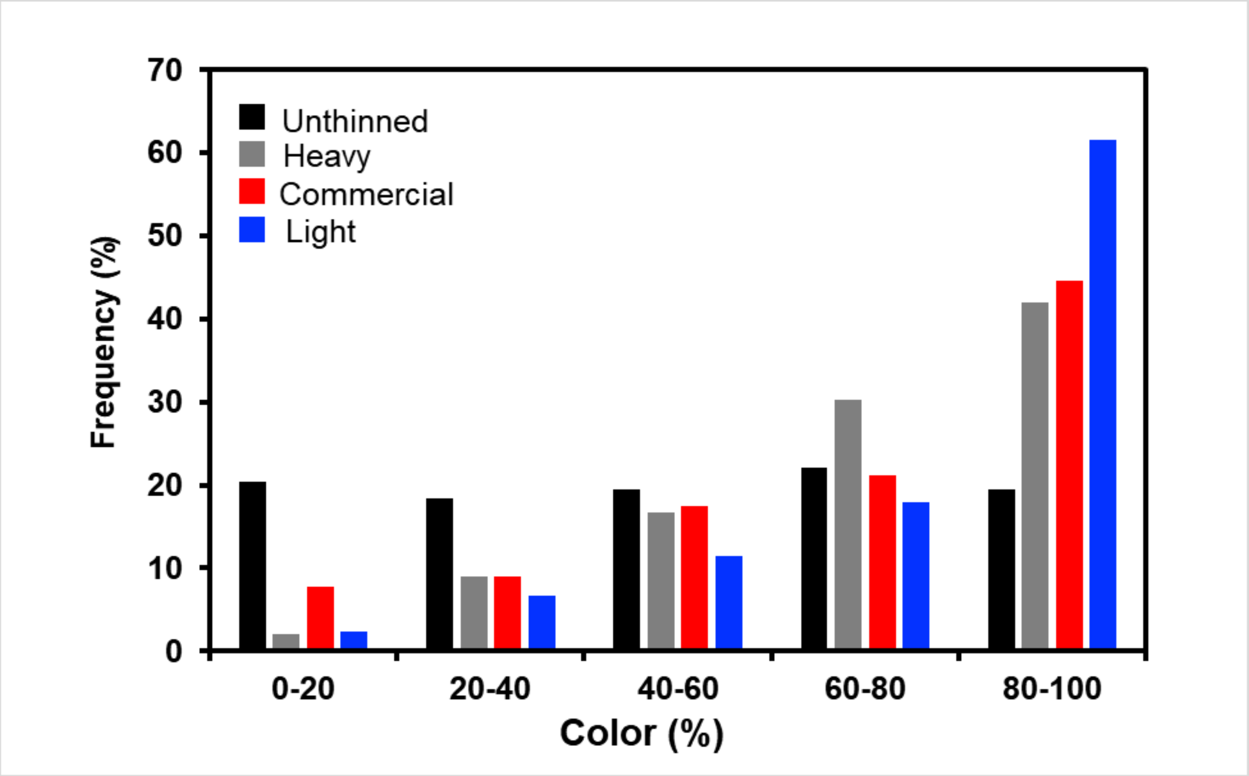

Figure 2.6. Effect of crop load on ‘Gala’ fruit red overcolor blush coverage (%) distribution at harvest. Fruit coming from ’Gala’ apple trees were unthinned and thinned to three different crop loads (heavy, commercial, and light crop loads) were measured for their red over color blush coverage at harvest. The effect of crop load on fruit red overcolor blush coverage distribution was determined in 100 fruit per tree and 3 trees per treatment and is presented as the percentage of fruits that are classified within different clusters of fruit red overcolor blush. The percentage of apple fruit that was classified in the clusters of more red overcolor blush coverage was increased by more thinning.

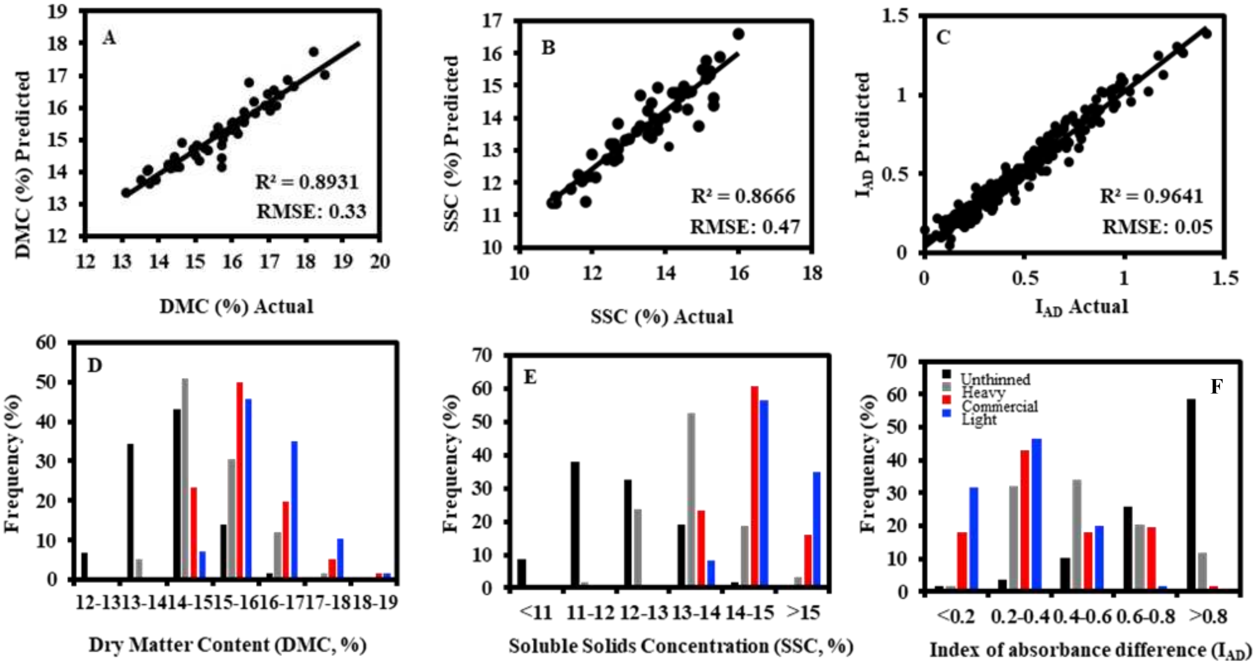

Figure 2.7. Non-destructive model validation and distribution of quality parameters at harvest. Fruit coming from 'Gala' apple trees were unthinned and thinned to three different crop loads (heavy, commercial, and light crop loads) were used to determine non-destructive fruit quality and maturity parameters using near infrared (NIR) spectroscopy at harvest (129 DAFB). To validate the quality and maturity parameters, the non-destructive predicted estimations of dry matter content (DMC) (A), soluble solids concentration (SSC) (B), and index of absorbance difference (I_{AD}) (C) were displayed against the actual destructive values. To show how well the developed models for these factors performed in assessing maturity and various quality metrics using near-infrared (NIR) spectroscopy, the models' linearity (R^2) and root mean square error of prediction (RMSEP) were examined (A- C). The effect of crop load on the distribution of the 'Gala' fruit was evaluated using non-destructive NIRS multivariate models and is illustrated (D-F).

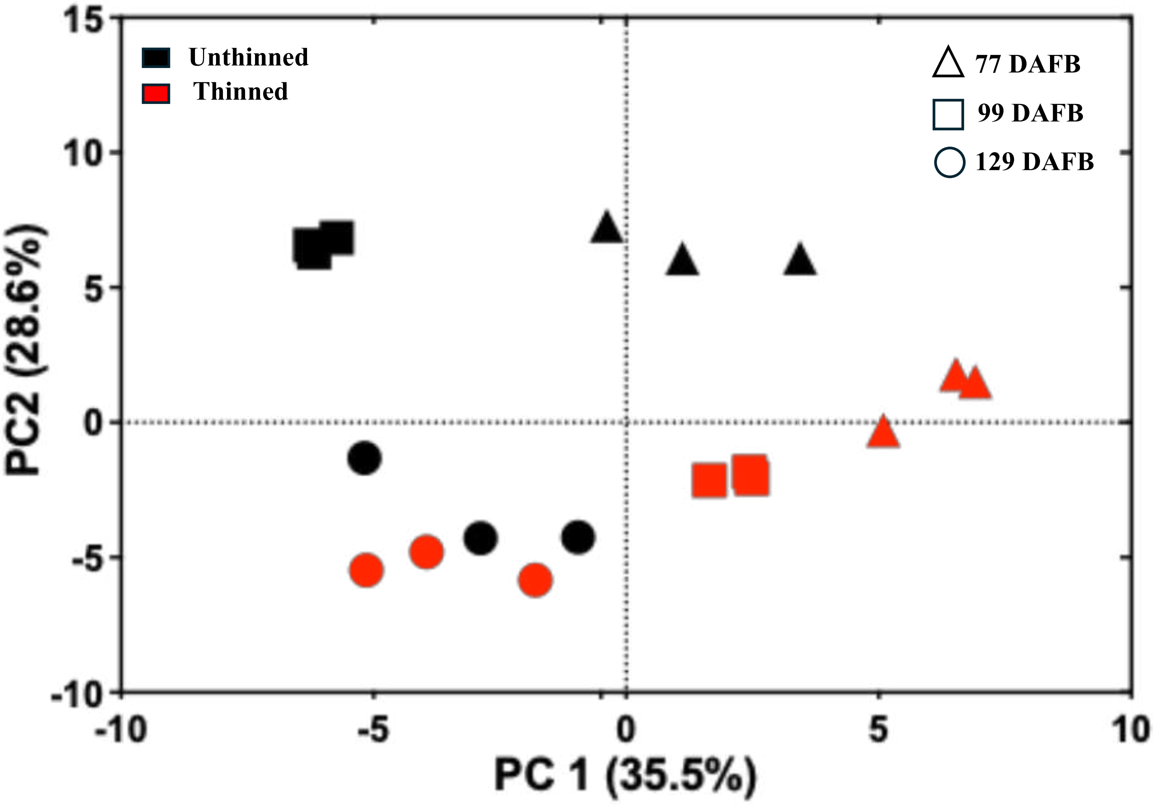

Figure 2.8. Principal component analysis of variable carbon competition treatments on apple fruit metabolism. Symbols indicate the scores for the thinning treatments (unthinned (black) vs. thinned (red)) and developmental stages (S2 (triangle), S3 (square), S4 (circle)) that are scaled with the abundance of primary metabolites detected in the apple cortex by non-targeted GC-MS analysis. Principal component analysis (PCA) of the three reps per thinning x developmental stage treatment demonstrates that developmental stage was a major contributor for metabolome variation as indicated by separation on PC 1 (~ 35.5%). Additional metabolome variation due to carbon availability and other factors are visualized on PC 2 (~ 28.6%). PCA shows wide variation in metabolome between carbon supply treatments at S2, and S3 while at S4, fruit metabolome scores are minimally different.

CHAPTER THREE

EFFECT OF CROP LOAD AND 1-METHYLCYCLOPROPENE (1-MCP) ON APPLE PERFORMANCE DURING POSTHARVEST STORAGE

1. Introduction

Loss of fruit quality can occur at any point throughout the supply chain, such as during harvesting, handling, storage, and transportation. Therefore, it is crucial to pay close attention to detail at all levels of the supply chain to preserve fruit quality (Nissen et al, 2018). Numerous research and field experiments conducted over the last 40 years indicate that 40-50% of horticulture crops in developing countries are lost before consumption, mostly due to elevated bruising, moisture loss, and subsequent deterioration during postharvest handling and many other disorders that could occur between the harvest and consumption (Kitinoja et al., 2002; Bekele, 2018). On the other hand, apples have potential for prolonged storage due to increased starch concentration in fruit tissues which are hydrolyzed into sugars to support respiration, thus delaying deterioration and allowing long storage and extended shelf life. The term postharvest apple quality refers to the state of apples after harvest, which includes the levels of fruit overcolor-blush, sweetness, firmness, texture, nutritional content and the presence or absence of any physiological disorders. For apple and most other fruits, quality improves only at the preharvest stage by the appropriate management of genetic and orchard factors and the occurring growing climate (Minas et al., 2018). The preharvest factors that affect the postharvest apple quality are genetic factors, fruit maturity at harvest, climatic factors, mineral nutrition, and cultural management practices (Thokar et al., 2022).

Many fruit quality attributes, including color, shape, size, weight, disease tolerance, as well as biochemical composition, are influenced by cultivar and rootstocks (Mussachi et al., 2018). Sensitivity to physiological disorders is one aspect that is significantly affected by genotypes. For example, sunburn sensitivity differs across cultivars, and this can affect the aesthetic and economic value of apples. Impacted fruits often exhibit a deterioration in quality at the time of harvest and during the postharvest period (Schrader et al., 2009).

Equally important, the use of rootstocks in modern tree fruit production has many benefits, including the scion vigor limitation, the enhancement of yield efficiency, and the creation of canopy architectural configurations that may lead to better fruit quality (Wang et al., 2019; Reighard et al., 2020; Anthony et al., 2021). In a study conducted with ‘Golden Delicious’ apples that were harvested at three picking times, the use of a dwarf (M9) and a semi-dwarf (MM111) rootstock resulted in fruit that had different harvest quality and maturity characteristics and behaved differently during postharvest storage. Apples grafted on MM111 rootstock stored better than apples grafted on M9 rootstock following the first pick date compared to the later picking times. However, following storage after the third harvest time MM111 fruit had the higher firmness, total sugars, total phenolic compounds, and antioxidant activity compared to M9 fruits. These results highlight the importance of rootstock selection and picking time or maturity at harvest for optimum postharvest performance (Nazari et al., 2024).

Fruit maturation is a genetically programmed process of fruit growth and development leading to the attainment of physiological maturity that determines seed reproductive capacity and is associated with fruit quality characteristics that meet consumer acceptance and at the same time ensure optimum postharvest performance. Apple maturity at harvest is the primary element that determines postharvest storage duration and final quality for the consumers. Optimum harvest timing is critical for all fruit growers including apple growers. Fruit picked at an immature stage is prone to shriveling and mechanical damage, while those harvested at an excessively mature stage have inferior texture, taste, and reduced storage and shelf life. Fruit picked prematurely or too late in the season are more susceptible to physiological disorders and have a lower shelf life compared to those harvested at optimal maturity. Consequently, harvesting at the optimal maturity stage is a critical factor for maintaining high levels of fruit quality and extending storage and shelf life (Bekele, 2018; Thokar et al., 2022). Maturity and quality indices that are traditionally used for decision making related to the timing of apple harvesting include fruit size, color, firmness, soluble solids concentration (SSC or °Brix), dry matter content (DMC) and starch content. Among all starch content or index is the standard parameter that the apple industry is using for harvest timing to make sure starch hydrolysis has started in the fruit, but the levels of the starch remaining are sufficient for prolonged postharvest storage. However, all these parameters are destructive and labor intensive and cannot be used for large data acquisition in the field (Musacchi et al., 2018; Minas et al., 2021).

Fruit or flower thinning is a crop load management practice that growers are performing to improve fruit quality by increasing the leaf-to-fruit ratio and reducing fruit-to-fruit competition for the available carbohydrates. Serra et al. (2016) indicated that careful crop load management is necessary to reduce biennial bearing and maintain yield consistency, while enhancing fruit quality and storability. Among the different preharvest factors, crop load adjustment by thinning has been one of the most significant parameters affecting fruit quality through the modification of carbohydrate availability during fruit growth and development. Crop load has been reported to affect apple fruit size, soluble and insoluble carbohydrate status, fruit color, dry matter content (DMC), soluble solid concentration (SSC), titratable acidity (TA), and chlorophyll content as measured by the index of absorbance difference (I_{AD}) (Musacchi et al., 2018).

During the postharvest and transport handling, fruit quality is greatly decreased by respiration, ripening, senescence, and decay. Low temperature and controlled atmosphere (CA) storage was traditionally the main postharvest storage strategy for apples (Kader et al., 2002). However, the need for specific infrastructure and expensive storage equipment has led growers to the adoption of newer technologies that reduce deterioration and extend storage and postharvest life. Among these technologies, 1-methylcyclopropene (1-MCP) has demonstrated the ability to prolong the shelf-life of fruits and vegetables by binding to the ethylene receptor and thereby significantly delaying the ripening process of climacteric fruit like apple. The use of 1-MCP as an ethylene action antagonist became popular among physiologists, horticulturists, food scientists, and postharvest technologists in the last 2 decades. 1-MCP, originally discovered by Sisler and Serek (1997), was commercially encapsulated into α-CD as the carrier material. The conventional application or delivery system of 1-MCP to fresh fruits and vegetables involves dissolving the inclusion complex into water or other solvents. 1-MCP gas is released into the atmosphere and interacts with fruits and vegetables. This conventional application had a great commercial success and impact in the apple industry and has become a standard postharvest technology.

The primary objective of this study was to examine the effect of crop load, the most important preharvest factor that influences fruit quality, on apple postharvest performance.

Previous research indicated that understanding pre harvest characteristics is essential for maintaining the optimum quality of apples during postharvest. The hypothesis of this research is that crop load can affect the carbohydrate status and the maturation process of the fruit which will affect fruit postharvest performance. Additionally, the effect of 1-MCP postharvest application was used as an industry standard treatment to allow the collection of relevant data.

2. Materials and methods

2.1. Plant material, crop load treatments, and postharvest treatments

'Gala' apples coming from four crop load treatments such as unthinned trees (1614 fruit/tree) or thinned to a heavy crop load (978 fruit/tree), commercial crop load (598 fruit/tree), and light crop load (380 fruit/tree) were harvested on August 19, 2016 from the Colorado State University's experimental orchard at the Western Colorado Research Center, Orchard Mesa (WCRC-OM) in Grand Junction, CO. The trees were managed, irrigated and fertilized based on the commercial standard practice of the region and 80 fruits per crop load (320 fruits in total), free from visual diseases and/or mechanical damage immediately after harvest, were randomly selected and divided into two postharvest treatments: (1) untreated control; and (2) treated with 1-MCP. Smart Fresh® tablets provided by AgroFresh, Inc. (Spring House, PA) were used as the source of 1-MCP (1.0 μL L^{-1}). Treated fruit were placed in a sealed tent in a cold room (0º C) and Smart Fresh® tablets were solubilized in distilled water and 1-MCP gas was circulated in the sealed tent with a fan, the fruits were exposed to 1-MCP for 24 h at room temperature. Shortly after treatment, fruits were cold stored at 0º C (95% relative humidity, RH), and separated into two additional groups: (1) fruits that were assessed for quality and ripening behavior after 3 months (m) of storage and additional shelf life and (2) quality and ripening behavior after 7 months of storage and additional shelf life. Both groups of untreated and 1-MCP treated fruit after every storage regime (3 or 7 m) were transferred to a ripening room (20°C, 90% RH), and apple ripening behavior and quality was characterized immediately at 0 days (d) and after 8 d.

2.2. Physiological characterization of postharvest ripening

Upon removal from cold storage, 40 fruit per treatment were put at 20ºC to acclimate under forced air conditioning using a fan for the internal temperature to reach 20 ºC within 2-3 hours. Twenty fruit per treatment were assessed at 0 d and twenty at 8 d during every shelf-life period. Fruit was assessed non-destructively for the index of absorbance difference (I_{AD}) and destructively for flesh firmness (FF), dry matter content (DMC), soluble solid concentration (SSC) and titratable acidity (TA). During shelf-life, flesh firmness (FF) was measured with a digital fruit texture analyzer (model FR-5120, Lutron Electronic Enterprise Co., Taipei, Taiwan) equipped with a 12-mm diameter stainless probe. Analysis was accomplished on a single side per fruit that was previously peeled with 1-mm thick skin tissue removed. Individual fruits were analyzed on opposite sides (sunny/shaded) at the equatorial region (Minas et al., 2021). Results corresponded with the maximum force applied by the probe to penetrate the fruit flesh by 10 mm expressed in newtons (N). Index of absorbance difference (I_{AD}) measurements were taken non-destructively with a handheld factory calibrated (closed type) Vis-NIRS sensor (DA-meter, T.R. Turoni srl, Forlì, Italy) (Costa et al., 2009). This index is used to evaluate the difference in absorbance between two light wavelengths (A670 nm - A720 nm), which are associated with the absorbance peaks of chlorophyll in the fruit surface and represent the background color or the physiological maturity of the fruit (Minas et al., 2021). DMC was measured destructively using a systematic sampling technique; samples were taken from the fruit cortex from opposite sides of each fruit using a 25-mm cork borer and the fresh weight of each sample was recorded, then the samples were dried in a forced air oven at 65°C until constant weight was reached after 3 days and then weighed to calculate the percentage (%) of dry matter in the fruit sample as follows: DMC (%) = [DW (g) / FW (g)]*100.

Soluble solid concentration (SSC, %) and titratable acidity (TA) were also analyzed destructively on longitudinal wedges (from the stem end to the blossom end) that were removed from each fruit, pooled to form 4 replicated composite samples (each coming from 5 fruit) and pressed through cheesecloth using a handheld juicer. First, a digital refractometer (PR 32, Atago, Tokyo, Japan) was used to calculate the SSC of fruit juice samples. Titratable acidity (TA) was determined in 5 mL of juice coming from bulked cortex tissue samples diluted in 50 mL of DI water. Titration was accomplished with 0.1 moL L^{-1} NaOH as the titrant that was poured through a digital burette (model Top Buret^{TM} M-25 mL, Eppendorf, Hauppauge, NY) to an inflection point of pH 8.2 which was determined with a pH meter (model SevenCompact S220, Mettler Toledo, Columbus, OH). Results were finally expressed as percentage of malic acid (%) as previously noted in Minas et al. (2013).

2.4. Statistical analysis

The statistical differences of all quality and maturity metrics between crop load and postharvest treatments during postharvest storage and shelf-life assessments have been evaluated using JMP Pro 13 software (JMP® 13 Automation Reference Copyright 2016, SAS Institute Inc., Cary, NC, USA). The difference in the fruit DMC, SSC, TA, FF, and I_{AD} mean values was evaluated using the student's t-test at (LSD, P = 0.05). It was employed to compare treatment means for specific ripening time points (0 d or 8 d).

3. Results and Discussion

3.1. Effect of crop load on apple postharvest quality and performance

The quality traits of the 'Gala' apple during postharvest were significantly influenced by crop load management applied during the preharvest stage. During the postharvest ripening after 3 or 7 months cold storage period, fruit coming from thinned trees retained better fruit quality and maturity parameters such as dry matter content (DMC), soluble solid concentration (SSC), titratable acidity (TA), flesh firmness (FF), and index of absorbance difference (I_{AD}) compared to the unthinned trees (Tables 3.1 - 3.4). Johnson (1992) came to an identical conclusion with the "Cox's Orange Pippin" apple where all thinning treatments greatly enhanced fruit size, post-storage FF, and DMC as compared to unthinned trees. The present research highlights that light crop load (380 fruit/tree) resulted in decreased yields (Chapter 2, Table 2.1), but retained better fruit quality characteristics (DMC, SSC, TA) and delayed fruit ripening (FF, I_{AD}) during the postharvest evaluation. In the next paragraphs the results of the postharvest performance of the fruit coming from different crop loads will be discussed in detail.

Dry matter content (DMC) refers to the components of a fruit that remain after the removal of water, such as sugars, starch, cell walls, organic acids, proteins, fibers, and minerals. DMC is becoming increasingly important in horticulture as it is linked to tree physiological performance in the orchard and is associated with consumer acceptance. Higher DMC results in elevated total carbohydrate levels, leading to improved fruit quality and consumer preference. During fruit growth, dry matter accumulates as sugars generated in nearby leaves are transported into the fruit via the phloem and converted to different soluble sugar forms or stored as starch. Sugars and starch, components of dry matter, gradually rise throughout fruit growth and development. Starch begins to hydrolyze to soluble sugars before harvest (Palmer et al., 2013; Palmer, 2014). DMC in apples is influenced by the cultivar, rootstock, pruning and training architecture, canopy position, and crop load. The harvest data in Chapter 2 showed that DMC increased with more thinning (Chapter 2; Table 2.2). Further, after 3 or 7 months of cold storage and subsequent shelf life, DMC was significantly higher in thinned treatments compared to unthinned trees (Table 3.1). Furthermore, if the DMC data at harvest was used as the benchmark dataset, DMC after 3 months and 0 days of storage (3m 0 d) decreased by 4.1% for the light crop load (CL) treatment, by 5.5% for the commercial crop load, by 1.3% for the heavy crop load, and by 3.3% for unthinned fruit. In addition, after 3m 8d of storage, DMC decreased by 1.8% for the light CL, by 6.7% for commercial CL, by 2.6% for the heavy CL, and by 0% for the unthinned CL. Similarly, after 7m (0 d and 8 d) of storage, the decreases on DMC were 4.2% and 1.2% for the light CL, 6.1% and 8% for the commercial CL, 3.9% and 3.2% for the heavy CL, and 0% and 1.4% for the unthinned CL after 0 and 8 days of storage, respectively. The observed loss in DMC is related to an indirect result of moisture loss through the transpiration of the fruit and the loss of carbon through respiration during prolonged storage and shelf life. Larger fruit coming from the thinned treatments are expected to lose higher levels of moisture, however, the ability of lighter crop loads to maintain significantly higher DMC levels during the postharvest period demonstrates the optimized physiological status of these fruit and the importance of this parameter to serve as an indicator of the ripe soluble solid concentration of the fruit when all starch is hydrolyzed (Palmer, 2014).

Soluble solids concentration is an industry standard fruit quality attribute that is related with sweetness for consumers. Soluble solids concentration or ^{o}Brix in fruit consist mostly of (~70%) of soluble sugars (e.g., fructose, glucose, and sucrose). The remaining portion represents organic acids, amino acids and soluble pectins. SSC increases during apple storage and shelf life, since starch that is abundant at harvest is hydrolyzed into sugars over time (Brookfield et al., 1997). Results showed that fruit coming from lighter crop loads had increased levels of SSC after cold storage (0°C and 95% RH) for 3 and 7 months, and subsequent ripening (up to 8 days, 20°C and 90% RH) mainly due to higher DMC or more starch that was hydrolyzed to soluble sugars (Table 3.1 - 3.4). Moreover, fruits coming from light crop load trees had high levels of SSC at harvest, which continued rising by 3.4% after 3 m of cold storage (3 m at 0°C and 0 d at 20°C) and by 4.8% following shelf-life simulation (8 d at 20°C). After 7 months of storage (0 d shelf life), the SSC in light crop load fruit increased by 1.4% and by 5.5% after 8 d shelf life. Similar results recorded with fruits coming from the commercial crop load treatment, where the amount of SSC at harvest was 14.1%, and this amount increased to about 14.5% after 3 m and 0 or 8 d and to 14.7% after 7 m and 0 or 8 d, were the increase rates were 2.8% and 1.4% for 3 m and 0 or 8 days and 4.3% for 7 m and 0 or 8 d of shelf life, respectively. Accordingly, with the results of the fruits which came from the heavy crop load and the unthinned trees, the levels of SSC at harvest were lower for both treatments, as shown in Chapter 2 (Table 2.2), than fruits coming from lighter crop loads, the levels of SSC increased by 9.8% and 5.2% for the heavy crop load after 3 m and 0 or 8 days, respectively. Interestingly, the levels of fruit coming from the heavy crop load SSC decreased during the shelf-life evaluation after 7 m by 6% and 2.3% for 0 and 8 d, respectively, when compared with their counterparts from the 3 m shelf-life period. In previous studies on a different apple cultivar (Honeycrisp'), it was reported that the levels of SSC increased after 1 month of storage in the lightest crop loads compared to the heaviest crop loads by 2% SSC, which agrees with what is reported herein (Serra et al., 2016).

Acidity in apples includes the total organic acid content, the most prominent of which is malic acid. Titratable acidity (TA, %) is often used as a measure of acidity and is important because it's effect on the eating experience (Kingston, 1992) and is also important for postharvest performance as it has been reported that higher values of TA correspond to a better storage performance (Watkins andNock, 2012). The results of the present study show that the levels of TA after 3m of storage (0 and 8 days) were significantly higher in fruit coming from the lowest crop loads (light and commercial crop loads) compared to fruit coming from higher crop loads (heavy crop load and unthinned trees) as it is illustrated in Tables 3.1 and 3.2 and Figure 3.2 (E and F) while the levels of TA decreased after 7 m of storage and subsequent shelf life (0 and 8 days) but still considered as high levels of TA for 'Gala' apple during postharvest handling. Prior research on different apple cultivars such as 'Jonagold' and 'Red Elstar' reported that there is no significant difference in TA among crop load treatments after storage (Awad et al., 2001). In addition, Serra et al. (2016) reported that there was no effect of crop load on 'Honeycrisp' TA after one month of storage, but after six months, the significant difference in TA shown on fruits coming from the lowest crop loads, were similar to what is reported in the current study.

Some of the most important attributes for apple consumers are the textural characteristics of apple fruit that reflect crispiness and is measured by flesh firmness (Brookfield et al., 2011). Low flesh firmness levels are one of the main reasons for postharvest apple quality loss, which could lower market demand by making customers less likely to purchase and accept apples (Johnston et al., 2002a; Harker et al., 2008). Consumers in a previous study viewed ‘Gala’ apples with a firmness below 45 N as too soft (Hoehn et al., 2003). The present study showed that apple fruits coming from lower crop loads maintained higher levels of firmness during storage. After 3 or 7 months at 0°C the FF levels were still high (79.6 N) for fruits that were harvested from the light crop load trees and even if they dropped by 19.7% compared to the FF levels at harvest (99.1 N). On the other hand, for the same postharvest storage period, FF dropped by 28.4% for fruits that had not been thinned (68.5 N). In addition, it was observed that higher FF levels were maintained in the lighter crop loads even after 8 days of ripening. Tables 3.1, 3.2, 3.3, and 3.4 display these results, as does Figure 3.2 (G and H). Saei et al. (2011) demonstrated that the apple FF levels were increased as the crop load decreased. The study by Volz et al. (2004) also found a link between lowering crop load and increased fruit firmness, which is similar to what other research has found for ‘Gala’ apples. However, the Cmelik et al. (2006) didn't find any evidence of higher fruit firmness levels with lowering crop load for the ‘Fuji’ cultivar. Our results align with the data reported by Delong et al. (2006) with 'Honeycrisp' apples. They observed that the mass of the fruit, firmness, soluble solids content (SSC), and titratable acidity (TA) all rose when the number of fruits per square centimeter of trunk cross-sectional area (TCSA) dropped, independently of the storage conditions or pre-storage treatments.

The nondestructive measurement of I_{AD} is a newer index that reflects the fruit's physiological maturity by measuring the chlorophyll content in the background of the fruit by using a handheld Vis-NIR spectrometer (DA-meter, T.R. Turoni srl, Forlì, Italy). The low values of this index indicate chlorophyll degradation during fruit maturation and ripening, while high values indicate more unripe fruit (Musacchi et al., 2018). I_{AD} was the only parameter that showed that crop load affected fruit maturity at harvest (see Chapter 2). During postharvest evaluations of the apples coming from different crop loads I_{AD} described better the variation in maturity as shown in Figure 3.2 (I and J) and in Tables 3.1 -3.4. Lower I_{AD} values for more physiologically mature fruit after more thinning were observed after 3 or 7 m of storage, and 0 and 8 days of shelf life. It is critical to highlight that an interesting result as reported in this study were fruit coming from commercial and light crop loads (even if they were more mature at harvest based on I_{AD} results, and because they had more DMC), exhibited less fruit softening as was indicated by FF. Compared with fruits from the heavy crop load and the unthinned trees that were harvested less mature, it would have been expected for the less mature fruit to soften slower. However, the opposite was observed, likely because fruit from the heavy crop load and unthinned trees had lower DMC levels, and possibly lower starch content to hydrolyze during storage, they softened and deteriorated faster. Thus, it is important to highlight that the internal quality at harvest is affecting firmness during postharvest. Based on the present FF data, if all crop load treatments were harvested at the same physiological maturity based on I_{AD} and with similar DMC differences, the FF retention levels after 3 or 7 months of storage should be even more pronounced.

3.2. The effect of crop load and 1-MCP on apple quality and postharvest performance

1-Methylcyclopropene (1-MCP) has been used effectively to preserve the quality of apples under commercial storage conditions. 1-MCP’s mode of action is blocking ethylene receptors, that reduces autocatalytic ethylene synthesis and respiration rates, inhibiting softening, and prolonging the storage and shelf life of climacteric fruit like apple (Minas et al., 2013; Mattheis, 2008). This study also highlights the significant effect of 1-MCP in maintaining the quality of ‘Gala’ apples during long-term cold storage. The beneficial effect of 1-MCP was observed in all quality (DMC, SSC, TA) and maturity (FF, I_{AD}) parameters tested across all storage durations and during shelf life (Tables 3.5 – 3.8) compared to the untreated fruit that discussed in the Section 3.1. (Tables 3.1 – 3.4). In terms of fruit quality (DMC, SSC, and TA), light and commercial crop loads treated with 1-MCP retained higher values of fruit quality than the heavy crop load and the unthinned trees at all four shelf lifetime points (3 m 0 d, 3 m 8 d, 7 m 0 d, and 7 m 8 d). In addition, after comparing these results with the results of fruit quality parameters at harvest it was found that 1-MCP inhibited the loss of DMC during storage and shelf life across all crop load treatments, compared to the untreated (control) with 1-MCP fruit, however, this was more pronounced in the light crop load fruit treated with 1-MCP that retained very high levels of DMC during storage and shelf life (Figures 3.3A and B). The reason for the inhibition of DMC loss is that 1-MCP reduces the levels of respiration rates during postharvest storage and shelf life of climacteric fruit. Furthermore, the application of 1-MCP caused the delay in the rise of soluble solids concentration (SSC) during ripening for 0 and 8 days following 3 and 7 months of cold storage across all crop load treatments (Figures 3.3C and D). In addition, there were significant noticeable differences in titratable acidity (TA) between fruits across all crop loads treated with 1-MCP and fruits untreated, especially after 7 m 8 d a significant decrease in TA was observed mainly untreated with 1-MCP fruit (Figures

3.2E and F and Figures 3.3E and F). The decrease in TA for the untreated with 1-MCP light, commercial and heavy crop load and unthinned trees was 61, 60, 51, and 59%, respectively following 7 months cold storage plus 8 days shelf life. While the fruit treated with 1-MCP for the same storage and shelf-life interval did not show a noticeable TA loss compared with the results of 3 m of cold storage plus 0 and 8 days ripening where the TA decrease of commercial, and heavy crop loads, and unthinned trees was 26, 24, 41, and 41%) respectively. Generally, these results agree with what Kolniak-Ostek et al. (2014) reported in their study on the effect of 1-MCP on two apple cultivars, Idared' and 'Shampion', where they found that prolonged storage of apples led to a higher DMC and delay in SSC increase and TA loss.

Regarding flesh firmness (FF) it was found that 1-MCP had a significant effect in FF retention during storage and shelf life maintaining a crispy texture in all treated fruit among all crop loads compared to the untreated fruit. 1-MCP treated fruit coming from lighter crop loads (light and commercial crop load) maintained the highest levels of FF during the postharvest storage and shelf-life periods (Figures 3.3G and H). These results highlight the significant impact of 1-MCP in apple postharvest physiology and agree with the characterization of this technology as a 'game changer' for the apple industry (Watkins, 2008). I_{AD} decreased by reducing crop load at harvest, however, the postharvest application of 1-MCP caused a higher retention of I_{AD} values across all crop load treatments during postharvest storage and shelf-life evaluation compared to the untreated treatments (Figures 3.2I and J and Figures 3.3I and J). It is worth mentioning that all crop loads treated with 1-MCP did not show any significant drop of I_{AD} during shelf life after both storage periods (Figures 3.3I and J) compared to the untreated fruits were mainly the heavy crop loads exhibited a significant decrease of I_{AD} during shelf life after both storage periods (Figures

3.2I and J). It is interesting to highlight that even though fruit coming from lighter crop loads were judged as more mature based on I_{AD} at harvest, this fruit following 1-MCP application or not still showed optimum storage performance during postharvest mainly because of the significantly higher DMC levels at harvest. These results were consistent with the Serra et al. (2016) report on 'Honeycrisp' where they concluded that fruit with longer storage life had better pre-storage quality because of optimum crop load management.

4. Conclusions

In this work, the quality traits of 'Gala' apple, as evaluated at different storage and shelf-life interval examinations, were significantly affected by the crop load adjustment by thinning applied on the apple trees during the preharvest period and more significantly by the pre-storage application of 1-MCP following harvest. Crop load not only affects fruit size and internal quality at harvest but also significantly affects apple fruit storage performance. Fruit coming from lighter crop loads had increased levels of SSC after postharvest ripening due to higher DMC levels at harvest. Fruit coming from lighter crop loads expressed lower softening rates during storage. Potentially, because of an increased carbohydrate pool that allows for a delayed deterioration. Higher DMC levels at harvest on fruit that was deemed even more mature based on I_{AD} to behave optimally throughout storage and shelf-life evaluations. In addition, 1-MCP when applied immediately after harvest can effectively inhibit fruit ripening through the suppression of respiration and ethylene production, delaying or stopping fruit softening, and loss of TA during postharvest as compared with untreated fruits. The fruit maintained higher DMC and SSC levels compared to untreated fruit due to reduced respiration levels. This effect in fruit quality preservation not only enhances the shelf-life performance of the fruit but also improves its marketability, ensuring that consumers receive fresher and more flavorful products. Consequently, the use of 1-MCP in combination with optimal crop load management and harvesting practices can significantly benefit both producers and consumers in the supply chain.

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Table 3.1. Effect of crop load on Gala apple fruit internal quality and maturity parameters after 3 months postharvest storage and 0 d shelf life. Following harvest, apples (cv. 'Gala') coming from different crop loads were cold stored at 0°C, RH 95% for 3 months. Fruits were removed from cold storage and transferred to 20°C (90% RH), where apple quality and ripening were characterized immediately. Changes in internal fruit quality as determined by dry matter content (DMC), soluble solid concentration (SSC), titratable acidity (TA) and maturity as determined by flesh firmness (FF) and index of absorbance difference (I_{AD}) were recorded after 3 months at 0 ºC and 0 days at 20 ºC.

Crop load	DMC (%)	SSC (%)	TA (%)	FF (N)	I_{AD} (%)
Unthinned	14.7 ± 0.3b	13.4 ± 0.2b	0.22 ± 0.01d	68.5 ± 3.8b	0.36 ± 0.06a
Heavy	15.3 ± 0.2ab	14.6 ± 0.3a	0.27 ± 0.01c	70.3 ± 2.1ab	0.25 ± 0.04b
Commercial	15.4± 0.3a	14.5± 0.2a	0.33 ± 0.01b	71.9 ± 2.3ab	0.16 ± 0.03b
Light	16.0 ± 0.2a	15.1 ± 0.5a	0.41 ± 0.02a	79.6 ± 1.3a	0.15 ± 0.02b

*Mean values in columns ± STD error followed by the same letter are not statistically significant according to Student's T test (*P*=0.05)

Table 3.2. Effect of crop load on Gala apple fruit internal quality and maturity parameters after 3 months postharvest storage and 8 d shelf life. Following harvest, apples (cv. 'Gala') coming from different crop loads were cold stored at 0°C, RH 95% for 3 months. Fruits were removed from cold storage and transferred to 20°C (90% RH), where apple quality and ripening was characterized for up to 8 d. Changes in internal fruit quality as determined by dry matter content (DMC), soluble solid concentration (SSC), titratable acidity (TA) and maturity as determined by flesh firmness (FF) and index of absorbance difference (I_{AD}) were recorded after 3 months at 0 ºC and 8 days at 20 ºC.

Crop load	DMC (%)	SSC (%)	TA (%)	FF (N)	I_{AD} (%)
Unthinned	14.2 ± 0.4c	13.5 ± 0.3c	0.20 ± 0.005c	56.7 ± 1.4b	0.24 ± 0.02a
Heavy	15.9 ± 0.9bc	14.0 ± 0.3bc	0.24 ± 0.006b	59.9 ± 1.9ab	0.17 ± 0.03b
Commercial	15.2 ± 0.3b	14.3 ± 0.2b	0.24 ± 0.002b	56.4 ± 1.7b	0.18 ± 0.02b
Light	16.4 ± 0.3a	15.3 ± 0.3a	0.33 ± 0.004a	64.9 ± 2.3a	0.07 ± 0.01c

*Mean values in columns ± STD error followed by the same letter are not statistically significant according to Student's T test (*P*=0.05)

Table 3.3. Effect of crop load on Gala apple fruit internal quality and maturity parameters after 7 months postharvest storage and 0 d shelf life. Following harvest, apples (cv. 'Gala') coming from different crop loads were cold stored at 0°C, RH 95% for 7 months. Fruits were removed from cold storage and transferred to 20°C (90% RH), where apple quality and ripening were characterized immediately. Changes in internal fruit quality as determined by dry matter content (DMC), soluble solid concentration (SSC), titratable acidity (TA) and maturity as determined by flesh firmness (FF) and index of absorbance difference (I_{AD}) were recorded after 7 months at 0 ºC and 0 days at 20 ºC.

Crop load	DMC (%)	SSC (%)	TA (%)	FF (N)	I_{AD} (%)
Unthinned	14.2 ± 0.2c	13.0 ± 0.2c	0.13 ± 0.00c	60.0 ± 1.7b	0.31 ± 0.02a
Heavy	14.9 ± 0.2bc	14.1 ± 0.2b	0.18 ± 0.00b	63.7 ± 1.4ab	0.23 ± 0.03b
Commercial	15.3 ± 0.3ab	14.7 ± 0.2ab	0.21 ± 0.02a	66.4 ± 2.4a	0.16 ± 0.02c
Light	16.0 ± 0.3a	14.8 ± 0.2a	0.24 ± 0.01a	68.8 ± 2.7a	0.11 ± 0.01c

*Mean values in columns ± STD error followed by the same letter are not statistically significant according to Student's T test (*P*=0.05)

Table 3.4. Effect of crop load on Gala apple fruit internal quality and maturity parameters after 7 months postharvest storage and 8 d shelf life. Following harvest, apples (cv. 'Gala') coming from different crop loads were cold stored at 0°C, RH 95%. Fruits were removed from cold storage and transferred to 20°C (90% RH), where apple quality and ripening was characterized for up to 8 d. Changes in internal fruit quality as determined by dry matter content (DMC), soluble solid concentration (SSC), titratable acidity (TA) and maturity as determined by flesh firmness (FF) and index of absorbance difference (I_{AD}) were recorded after 7 months at 0 ºC and 8 days at 20 ºC.

Crop load	DMC (%)	SSC (%)	TA (%)	FF (N)	I_{AD} (%)
Unthinned	14.4 ± 0.3b	13.2 ± 0.3b	0.09 ± 0.005c	49.0 ± 3.0a	0.18 ± 0.02ab
Heavy	15.0 ± 0.2b	13.6 ± 0.1b	0.13 ± 0.007b	46.9 ± 2.7ab	0.21 ± 0.02a
Commercial	15.0 ± 0.2b	14.7 ± 0.3a	0.13 ± 0.004b	39.7 ± 2.8b	0.17 ± 0.02ab
Light	16.5 ± 0.3a	15.4 ± 0.4a	0.16 ± 0.002a	41.8 ± 1.6ab	0.13 ± 0.01b

*Mean values in columns ± STD error followed by the same letter are not statistically significant according to Student's T test (*P*=0.05)

Table 3.5. Effect of crop load and 1-MCP on ‘Gala’ apple fruit internal quality and maturity after 3 months postharvest storage and 0 d shelf life. Following harvest apples (cv. ‘Gala’) coming from different crop loads were treated with 1-MCP (1.0 µL L^{-1} 0°C, 24 h) and then cold-stored (0°C, RH 95%) for 3 months. Fruits were removed from cold storage and then transferred to 20°C (90% RH), where apple quality and ripening were characterized immediately. Changes in internal fruit quality as determined by dry matter content (DMC), soluble solid concentration (SSC), titratable acidity (TA) and maturity as determined by flesh firmness (FF) and index of absorbance difference (I_{AD}) were recorded after 3 months at 0 ºC and 0 days at 20 ºC.

Crop load	DMC (%)	SSC (%)	TA (%)	FF (N)	I_{AD} (%)
Unthinned	14.2 ± 0.3b	13.3 ± 0.3c	0.26 ± 0.00b	80.1 ± 1.7a	0.28 ± 0.04a
Heavy	15.9 ± 0.4a	14.8 ± 0.2b	0.30 ± 0.01b	83.4 ± 3.5a	0.20 ± 0.03a
Commercial	16.0 ± 0.5a	14.8 ± 0.3b	0.38 ± 0.02a	84.8 ± 3.3a	0.21 ± 0.03a
Light	16.4 ± 0.2a	15.6 ± 0.2a	0.41 ± 0.03a	83.1 ± 3.6a	0.10 ± 0.02b

*Mean values in columns ± STD error followed by the same letter are not statistically significant according to Student’s T test (*P*=0.05)

Table 3.6. Effect of crop load and 1-MCP on 'Gala' apple fruit internal quality and maturity after 3 months postharvest storage and 8 d shelf life. Following harvest apples (cv. 'Gala') coming from different crop loads were treated with 1-MCP (1.0 µL L^{-1} 0°C, 24 h) and then cold-stored (0°C, RH 95%) for 3 months. Fruits were removed from cold storage and transferred to 20°C (90% RH), where apple quality and ripening was characterized for up to 8 d. Changes in internal fruit quality as determined by dry matter content (DMC), soluble solid concentration (SSC), titratable acidity (TA) and maturity as determined by flesh firmness (FF) and index of absorbance difference (I_{AD}) were recorded after 3 months at 0 ºC and 8 days at 20 ºC.

Crop load	DMC (%)	SSC (%)	TA (%)	FF (N)	I_{AD} (%)
Unthinned	15.2 ± 0.3c	14.1 ± 0.3c	0.26 ± 0.01c	74.4 ± 2.1a	0.40 ± 0.04a
Heavy	15.2 ± 0.2c	14.3 ± 0.2c	0.33 ± 0.00b	78.9 ± 2.9a	0.26 ± 0.02b
Commercial	16.1 ± 0.3b	15.2 ± 0.2b	0.35 ± 0.01b	79.8 ± 2.7a	0.21 ± 0.02bc
Light	17.0 ± 0.3a	16.0 ± 0.1a	0.40 ± 0.01a	81.9 ± 3.3a	0.18 ± 0.02c

*Mean values in columns ± STD error followed by the same letter are not statistically significant according to Student's T test (*P*=0.05)

Table 3.7. Effect of crop load and 1-MCP on 'Gala' apple fruit internal quality and maturity after 7 months postharvest storage and 0 d shelf life. Following harvest apples (cv. 'Gala') coming from different crop loads were treated with 1-MCP (1.0 µL L^{-1} 0°C, 24 h) and then cold-stored (0°C, RH 95%) for 7 months. Fruits were removed from cold storage and then transferred to 20°C (90% RH), where apple quality and ripening were characterized immediately. Changes in internal fruit quality as determined by dry matter content (DMC), soluble solid concentration (SSC), titratable acidity (TA) and maturity as determined by flesh firmness (FF) and index of absorbance difference (I_{AD}) were recorded after 7 months at 0 ºC and 0 days at 20 ºC.

Crop load	DMC (%)	SSC (%)	TA	FF (N)	I_{AD}
Unthinned	14.7 ± 0.3b	13.3 ± 0.2c	0.27 ± 0.01b	72.5 ± 2.3a	0.31 ± 0.03a
Heavy	15.9 ± 0.3a	14.5 ± 0.3b	0.31 ± 0.01ab	78.0 ± 2.9a	0.24 ± 0.02b
Commercial	16.5 ± 0.6a	15.3 ± 0.3ab	0.28 ± 0.03b	74.5 ± 3.3a	0.16 ± 0.02c
Light	16.9 ± 0.2a	15.6 ± 0.3a	0.34 ± 0.01a	77.7 ± 3.7a	0.12 ± 0.02c

*Mean values in columns ± STD error followed by the same letter are not statistically significant according to Student's T test (*P*=0.05)

Table 3.8. Effect of crop load and 1-MCP on 'Gala' apple fruit internal quality and maturity after 7 months postharvest storage and 8 d shelf life. Following harvest apples (cv. 'Gala') coming from different crop loads were treated with 1-MCP (1.0 µL L^{-1} 0°C, 24 h) and then cold-stored (0°C, RH 95%) for 7 months. Fruits were removed from cold storage and transferred to 20°C (90% RH), where apple quality and ripening was characterized for up to 8 d. Changes in internal fruit quality as determined by dry matter content (DMC), soluble solid concentration (SSC), titratable acidity (TA) and maturity as determined by flesh firmness (FF) and index of absorbance difference (I_{AD}) were recorded after 7 months at 0 ºC and 8 days at 20 ºC.

Crop load	DMC (%)	SSC (%)	TA	FF (N)	I_{AD}
Unthinned	15.3 ± 0.4c	14.6 ± 0.3c	0.23 ± 0.1b	75.2 ± 3.3b	0.37 ± 0.04a
Heavy	16.2 ± 0.2b	15.1 ± 0.3bc	0.16 ± 0.01c	75.5 ± 2.2b	0.26 ± 0.02b
Commercial	16.4 ± 0.3b	15.5 ± 0.2b	0.25 ± 0.01b	70.6 ± 3.6b	0.21 ± 0.02b
Light	17.7 ± 0.2a	16.6 ± 0.2a	0.30 ± 0.01a	88.0 ± 4.2a	0.19 ± 0.02b

*Mean values in columns ± STD error followed by the same letter are not statistically significant according to Student's T test (*P*=0.05)

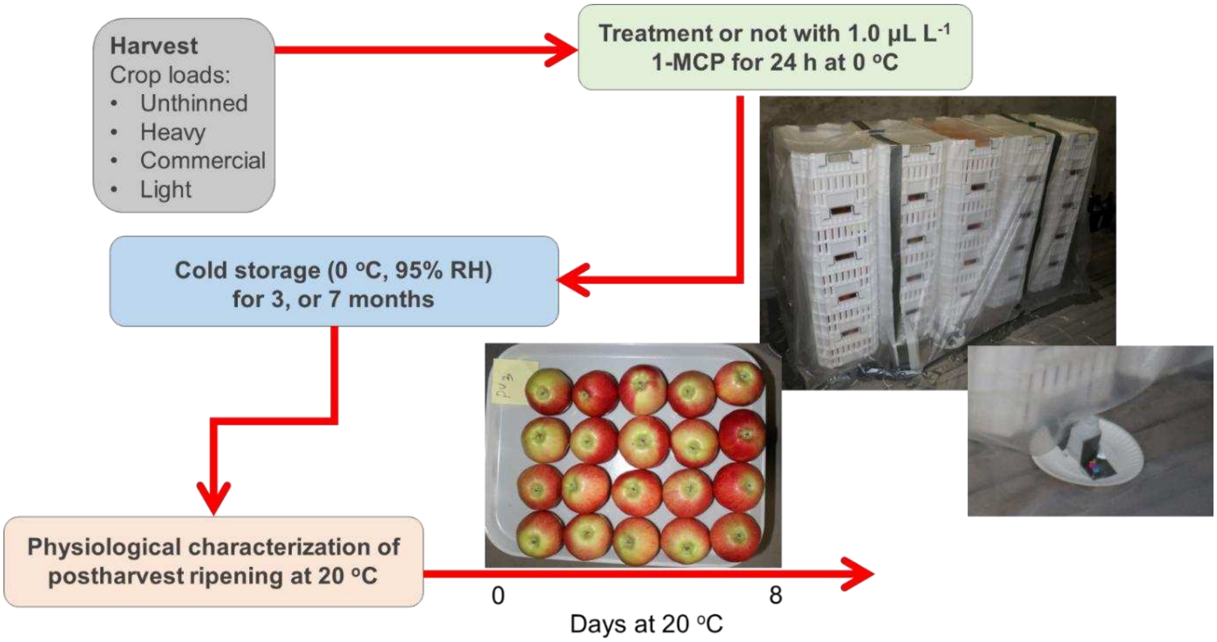

Figure 3.1. The experimental approach. The impact of crop load and 1-MCP on postharvest apple fruit quality. The fruit samples were divided into two groups: untreated fruits and those treated with 1-MCP. The fruits were exposed to 1-MCP for 24 hours, then cold storage for 3 and 7 months. After each storage period, half of the fruits were moved to a ripening room for a shelf-life evaluation for up to 8 days.

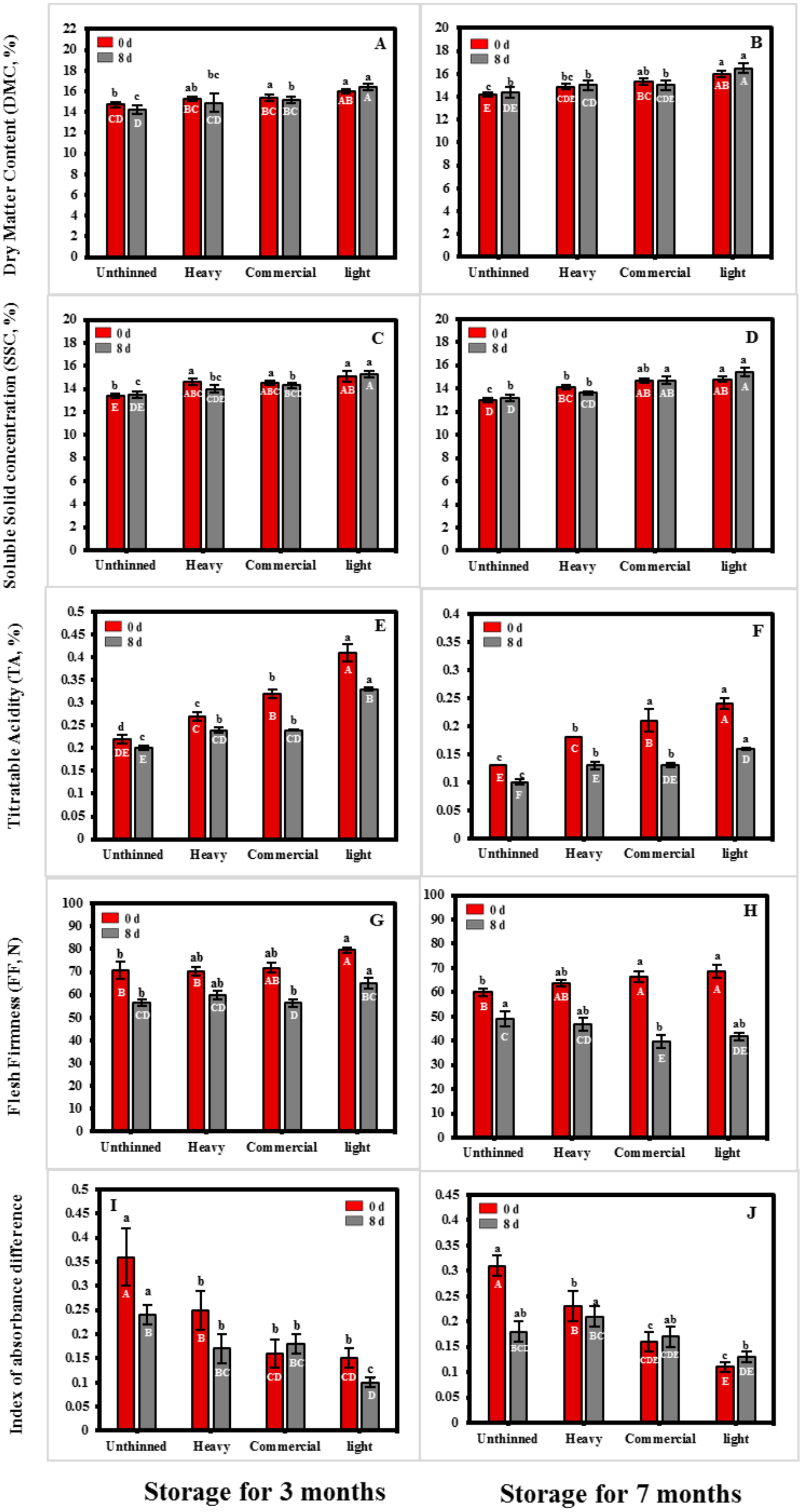
Dry Matter Content (DMC, %)
Soluble Solid concentration (SSC, %)
Titratable Acidity (TA, %)
Flesh Firmness (FF, N)
Index of absorbance difference
0 d
8 d
A
b CD c D ab BC bc CD a BC b BC a AB a A
0 2 4 6 8 10 12 14 16 18 20 22
B
c E b DE bc CDE b CD ab BC b CDE a AB a A
0 2 4 6 8 10 12 14 16 18 20
C
b E c DE a ABC bc CDE a ABC b BCD a AB a A
0 2 4 6 8 10 12 14 16 18 20
D
c D b D b BC b CD ab AB a AB a AB a A
0 2 4 6 8 10 12 14 16 18 20
E
d DE c E c C b CD b B b CD a A a B
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5
F
c E c F b C b E a B b DE a A a D
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4
G
b B b CD ab B ab CD ab AB b D a A a BC
0 10 20 30 40 50 60 70 80 90 100
H
b B a C ab AB ab CD a A b E a A ab DE
0 10 20 30 40 50 60 70 80 90 100
I
a A a B b B b BC b CD b BC b CD c D
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5
J
a A ab BCD b B a BC c CDE ab CDE c E b DE
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45
Unthinned
Heavy
Commercial
light
Storage for 3 months
Storage for 7 months

Figure 3.2. Effect of crop load on apple fruit quality and maturity parameters during postharvest shelf-life storage. Following harvest, apples (cv. 'Gala') coming from different crop loads were cold stored at (0°C, RH 95%) for up to 3 and 7 months. Following the cold storage period fruits were transferred to 20°C (90% RH), where apple quality ripening was characterized immediately (0 d) or after 8 d. Changes in DMC, SSC, TA, FF, and I_{AD} were determined. Values represent the average of 4 replicates of 5 fruits each that were analyzed at each ripening time point. Bars of the same color in figure plates followed by the same letter are not statistically significant according to Student's t-test (LSD, $P = 0.05$), which was used for comparisons of means between treatments for specific ripening time points. Error bars are standard error of the mean.

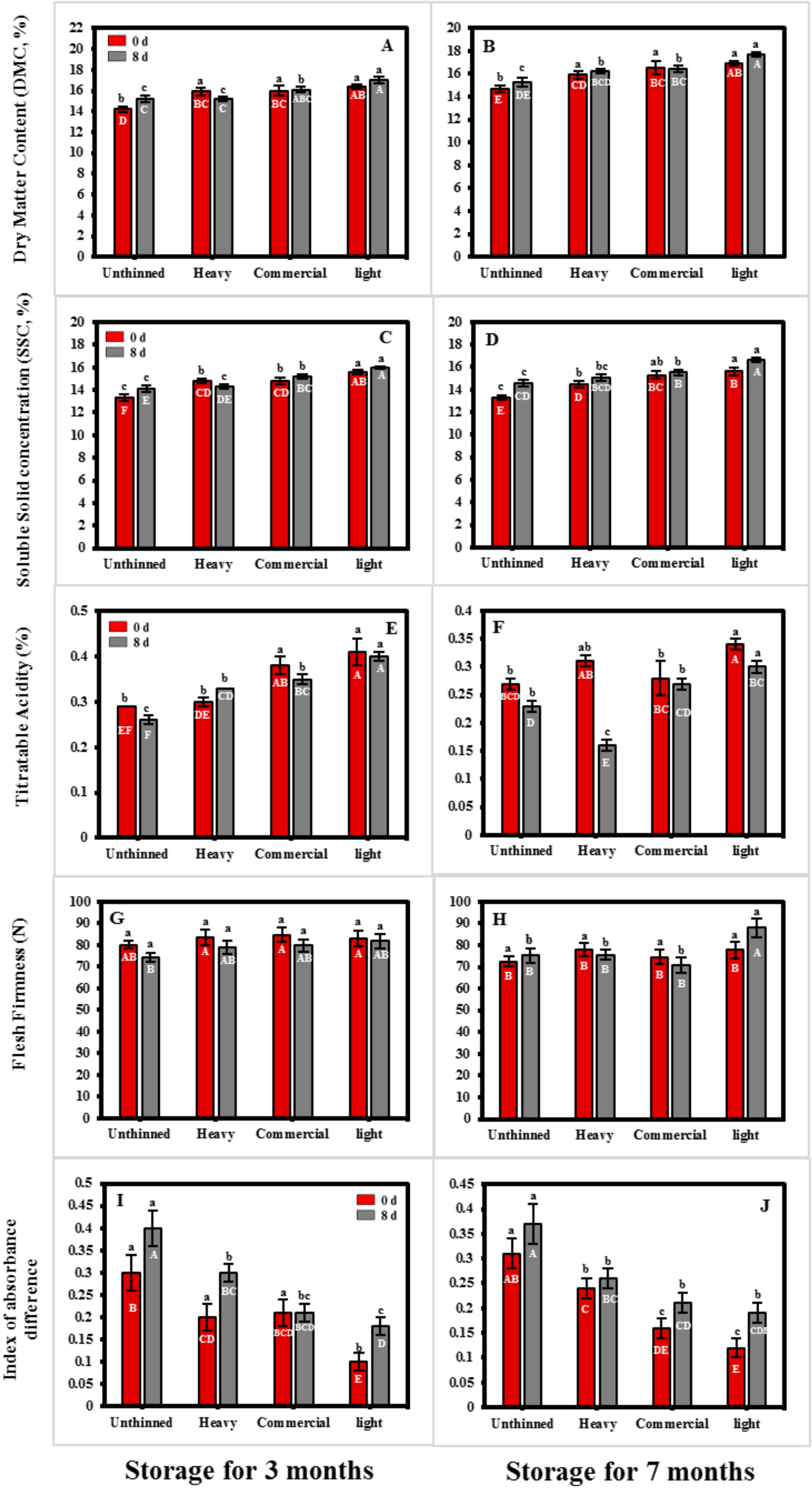
Dry Matter Content (DMC, %)
Soluble Solid concentration (SSC, %)
Titratable Acidity (%)
Flesh Firmness (N)
Index of absorbance difference
A
B
C
D
E
F
G
H
I
J
0 d
8 d
Unthinned
Heavy
Commercial
light
Storage for 3 months
Storage for 7 months

Figure 3.3. Effect of crop load and 1-MCP on apple fruit quality and maturity during postharvest shelf life after storage. Following harvest, apples (cv. 'Gala') coming from different crop loads were treated with 1-MCP (1.0 µL L^{-1}, 0°C, 24 h) and then cold-stored (0°C, RH 95%) for up to 3 or 7 months. Following the cold storage period fruits were transferred to 20°C (90% RH), where apple quality and ripening was characterized immediately (0 d) for after 8 d. Changes in DMC, SSC, TA, FF, and I_{AD} were determined. Values represent the average of four replicates of 5 fruits each that were analyzed at each ripening time point. Bars of the same color in figure plates followed by the same letter are not statistically significant according to Student's t-test (LSD, *P* = 0.05), which was used for comparisons of means between treatments for specific ripening time points. Error bars are standard error of the mean.