MAINTENANCE OF CARBON 7 SUGAR LEVELS AND EFFECT ON RIPENING OF 'FUERTE' AND 'HASS' AVOCADO (*Persea americana* Mill.) FRUIT

By

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ABSTRACT

Avocado fruit are susceptible to a large variety of disorders. These disorders may be a result of an inability of the mesocarp tissue to counteract or tolerate postharvest stress. The C7 sugars *D*-mannoheptulose and perseitol have been reported to form the predominant portion of antioxidants in the mesocarp and their presence has been associated with avocado fruit quality. It was, therefore, investigated, if mesocarp C7 sugar levels, particularly of *D*-mannoheptulose and perseitol, can be maintained through infusion of these sugars and further, if this C7 sugar level is associated with fruit quality and shelf life. Avocado fruit, harvested from 'Hass' and 'Fuerte' avocado orchards in the KwaZulu-Natal Midlands in three different season (early, middle, and late harvest) were infused with 1.5 mL water, 1.5 mL solution of (9.5 mM/fruit; 4.75 mM/fruit *D*-mannoheptulose), a C7 sugar solution (1.5 mL of 9.5 mM/fruit; 4.75 /fruit; 4.75 mM perseitol/fruit). Fruit quality parameters (firmness, CO_2 production, soluble sugar concentrations, moisture content, dry matter, and oil content) were determined over the postharvest ripening period. Early-harvested fruit displayed more severe ripening heterogeneity, with high water loss. The infusion of *D*-mannoheptulose and perseitol prolonged the shelf life of avocado fruit compared to sucrose-infusion and untreated fruit (control) at different harvesting stages. Water infusion had a considerable effect on mid- and late-season fruit, regarding firmness and respiration rate. Infusion of *D*-mannoheptulose and perseitol improved the fruit quality attributes flesh firmness and fresh mass retention, and resulted in higher mesocarp C7 sugar concentrations than sucrose- and water-infusion. Regarding the concentration of C7 sugars, water-infused fruit contained the third-highest *D*-mannoheptulose and perseitol concentration. The oil content was not affected by sugar postharvest infusion, but noticeable differences in oil content were observed through the harvest seasons. Maintaining a certain level of these sugars in the avocado mesocarp tissue seems vital in ensuring a good fruit

quality. These C7 sugars could be used as postharvest markers and determining their concentration could become a vital tool in the management of avocado postharvest quality.

Keywords: *avocado, sugar infusion, harvest season, C7 sugar, ripening*

COLLEGE OF AGRICULTURE ENGINEERING AND SCIENCE DECLARATION 1-PLAGIARISM

The research work reported in this thesis was as a result of experiments carried out in Horticultural Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Prof. Isa Bertling and Dr. Samson Tesfay (School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal; South Africa).

By submitting this thesis electronically, I hereby declare that the entirety of the research was as a result of my own investigations. It therefore represents my original work except where otherwise stated and due acknowledgments are accorded.

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We certify that the above statement is correct.

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Prof. Isa Bertling

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Dr. Samson Tesfay

Co-supervisor

GENERAL OVERVIEW

This study attempted to investigate, if the simulation of the fruit being attached to the tree through infusion of water, sucrose and the C7 sugars *D*-mannoheptulose or perseitol can delay the ripening process and if the onset of fruit softening can be delayed by this infusion. In this study the two C7 sugars *D*-mannoheptulose and its reduced polyol form, perseitol, were investigated as markers of postharvest quality of 'Fuerte' and/or 'Hass' avocado fruit. Although C7 sugars have been researched intensively in association with avocado postharvest quality, no literature has reported on the successfully maintenance of a certain size seven carbon sugar pool and its potential correlation with fruit quality. Infusion of C7 sugars into avocado fruit and utilization of a destructive method allows the measurement of the actual C7 sugar concentration as well as the maturity status of single fruit with respect to a specific parameter, and will allow determination of fruit acceptability to the consumer. The carbon 7 sugars found in avocado mesocarp tissue are well-known for their antioxidant activity, but the potential of these uncommon sugars to serve as biomarkers of postharvest avocado fruit quality has not been investigated. This study is divided into four sections, including two experimental chapters following the first chapter which reviews the literature available for understanding the role of common C6 sugars and the C7 sugars on fruit development and in the softening process. The second chapter is the first experimental chapter, evaluating the effect of C7 sugar (*D*-mannoheptulose or perseitol) or C6 sugar (sucrose) infusion as well as water infusion on the ripening pattern of 'Hass' and 'Fuerte' fruit of different maturity stages as defined by picking seasons (early-, mid-, and late-season fruit). The third chapter is the second experimental one, which investigates the effect of C7 sugar infusion on mesocarp sugar concentrations and oil content of individual fruit of different maturity stages as defined by picking seasons (early-, mid-, and late-season fruit). This is followed by a general discussion and conclusions; finally, future research prospects will be suggested.

COLLEGE OF AGRICULTURE ENGINEERING AND SCIENCE DECLARATION 2- PUBLICATIONS

LIST OF CONFERENCE PRESENTATION FROM THIS THESIS

1. **S. Mathe** and I. Bertling 2016. Infusion of seven carbon (C7) sugars to serve as markers for postharvest quality of 'Fuerte' and 'Hass' avocado (*Persea americana* Mill.) Combined Congress. University of Free State, Bloemfontein, 20-23 January, 2016. (Oral)

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PREFACE

This thesis is a compilation of manuscripts where each individual chapter is an independent article introduced disjointedly. Hence, some repetition between individual chapters has been inevitable.

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CHAPTER 1

1.1 GENERAL INTRODUCTION

The avocado (*Persea americana* Mill.) is an evergreen tree that develops flowers in spring on the previous summer growth tips (Scroeder, 1951; Whiley *et al.,* 1988). Flowering and fruit set of 'Hass' avocado in the KwaZulu-Natal Midlands takes place during the cooler month of July, but earlier cultivars, such as 'Fuerte', can start to flower in February, when it is relatively hot, with flowering proceeding until July, when temperatures are cooler (Kaiser and Wolstenholme, 1994). The 'Hass' cultivar is a late-maturing cultivar; it is said to have a higher oil percentage at maturity and a taste 'creamier than other cultivars' (Wood, 1984). As 'Hass' skin colour changes from green to black (Cox *et al.,* 2004), some of the fruit glossiness is lost; this change towards a duller fruit is an indication that the fruit is ready for consumption (Wood, 1984). In warmer areas of South Africa, 'Hass' matures at the beginning of August. This cultivar can resist bruising and withstand rough handling, due to thickness of the exocarp. From a producer perspective, 'Hass' has the advantage to be able to be 'stored on the tree', as the fruit does not abscise naturally when the point of commercial harvest, which is dictated by the mesocarp oil (or the reciprocal value water) content, is approaching. 'Hass' is, therefore, often 'stored on the tree' until market conditions are favorable (Wood, 1984), since it may be left on the tree without deterioration of fruit quality (Dodd *et al.,* 2010); however, fruit of the newer cultivar 'Lamb Hass' tend to be more prone to post-harvest physiological disorders than 'Hass' fruit, particularly during the late picking season (Dixon *et al.,* 2008).

Avocado has a high perishability and a relatively short shelf life (Jeong *et al.,* 2002); shelf life depends on cultivar and stage of maturity; harvesting immature avocados can result in economic losses due to poor fruit quality, such as shrivel upon storage, with the fleshy mesocarp becoming 'rubbery' rather than 'buttery' and stringy vascular tissue (Kader, 1999; Pak *et al.,* 2003). Avocado growers tend, however, to pick fruit early, sometimes too early and

immature fruit are picked in order to reach the early market price advantage (Sudheer and Indira, 2007). It is well-known that as long as the avocado fruit is attached to the tree, and the flow of inhibitive components from the leaves to the fruit, preventing fruit ripening on the tree or until their pedicels are girdled (Schroeder, 1953, Tingwa and Young, 1974, Lee *et al.,* 1983, Liu *et al.,* 2002). The rise in avocado popularity has forced avocado growers and importers to solve postharvest disorder problems that are related to the control of ripening and the prolonged fruit storage (Donetti, 2011). The C7 sugars in avocado are known to play a role in its unusual ripening habit, and this sugars are associated with fruit quality (Bertling and Bower, 2005; Cowan, 2004). Additionally, the C7 sugars concentration in the fruit mesocarp tends to decrease with fruit maturity (Bertling and Bower, 2005), although fruit maturity increases, while the fruit remains on the tree. Some biochemical changes occurring during avocado fruit development and fruit ripening suggest that the degree of ripening inhibition is also affected by fruit maturity (Tesfay *et al.,* 2010). As the harvesting season progresses, dry matter and oil accumulate; however, water content, C7 sugars, and mesocarp phenolics decrease (Pearson, 1975, Tesfay *et al.,* 2010, Tesfay *et al.,* 2011). The pathway regulating ripening seems to extend to other pathways, such as ethylene synthesis, which has made the detection of the mode of action of ripening rather unclear.

Avocado is known to contain C7 sugars in various plant parts and fruit parts, and commonly in greater amounts than the C6 sugars fructose, glucose and sucrose. The heptoses include the common Calvin cycle sugar sedoheptulose, as well as the uncommon C7 sugar aldoses mannoheptulose and the sugar alcohols perseitol (Häfliger *et al.,* 1999; Liu *et al.,* 2002; Tesfay *et al.,* 2010). The C7 sugar mannoheptulose commonly occurs in avocado leaves during photosynthesis (Liu *et al.,* 2002); however, the way in which Calvin cycle intermediates are starting points for the synthesis of mannoheptulose, or in which compartment of the leaf cell

this assembly takes place, is not clear (Tesfay *et al.*, 2010). It has also been suggested that postharvest fruit quality is associated with presence of C7 sugars (Bertling and Bower, 2005), and it has been demonstrated that the fruit sugar content varies between cultivars and growing regions (Kaluwa, 2010; Landahl *et al.*, 2009a). Van Zyl and Ferreira (1995) reported that postharvest, avocado fruit are very prone to quality loss, particularly, if exposed to ethylene, as raised ethylene production can increase the risk of physiological disorders and, therefore, enhance fruit quality loss. Avocado fruit are harvested based on visual attributes (size and colour) once they are of a certain oil or water content (Werman and Neeman, 1987). There is a lack of a specific physiological ripening parameter in avocado. During softening of many fruit the transformation into a palatable product occurs through a multitude of changes in fruit composition, such as the accumulation of volatiles, the synthesis and degradation of pigments and changes in the concentration of sugars and organic acids (Giovannoni, 2004; González-Agüero *et al.*, 2016; Obenland *et al.*, 2009). In many fruit species, such as apples, grapes and tomatoes, there are organoleptic quality characteristics associated with ripening and softening of the fruit (Crisosto *et al.*, 2003; Sweetman *et al.*, 2009). In avocado, although mesocarp oil or water content are used commercially as quality parameters, it is not uncommon, to find immature fruit being sold, as no physiological maturity parameter seems to exist. It is known, however, that mesocarp sugar concentrations change during ripening; but it is still unknown, if and how these changes are related to fruit maturity. This study, therefore, intended to determine whether the influx of C7 sugars can extend the days to ripening and reduce softening. The focus on *D*-mannoheptulose and perseitol was due to these being the most-abundant sugars and that they have been reported to become depleted during avocado ripening (Gamble *et al.*, 2010; Tesfay *et al.*, 2010).

1.2 RESEARCH HYPOTHESIS

For many fruit, it is difficult to identify the point of horticultural maturity, and avocado is no exception. There are no apparent changes in external fruit appearance that mark avocado maturity, indicating the time frame when the fruit can be harvested. Moreover, physiological mature avocado fruit do not soften on the tree, but such softening occurs only several days after being picked (Adato and Gazit, 1974). The time to fruit softening after picking depends on the stage of maturity, with less time required as fruit maturity increases (Adato and Gazit, 1974; Zauberman and Schiffmann-Nadel, 1972). In avocado, softening is triggered after harvest through the breakdown of cell walls by enzymes that cause softening and thereby edibility (Colinas-Leon and Young, 1981). While Blakey *et al.* (2009) reported that a certain fruit water content at harvest is critical to initiate the softening process, the authors also found a 70% variation in this parameter. The C7 sugar mannoheptulose compound has been reported as major contributor to the antioxidant activity of the mesocarp (Tesfay *et al.,* 2010), followed by its isomer perseitol at the time of picking maturity (Tesfay *et al.*, 2010). The C7 sugars, particularly *D*-mannoheptulose could play an important role protecting the mesocarp from various postharvest storage disorders. Therefore, the decrease in C7 sugars particularly *D*-mannoheptulose, is associated with postharvest losses, artificial supply, via infusing fruit with these substances, should maintain high postharvest quality. Therefore, fruit of the early-, mid-, and late-season were infused, through the pedicel to maintain a high C7 sugar concentration within the mesocarp to avoid mesocarp quality deterioration. This study attempts to investigate, if simulating that fruit remain attached to the tree (through infusion of water, sucrose or the C7 sugar *D*-mannoheptulose or perseitol) can retard fruit softening. The hypothesis is that maintaining a physiological *D*-mannoheptulose or perseitol concentration and water content in the fruit mesocarp delays fruit ripening and thereby enhances postharvest life.

1.2.1 Research aim

1.2.1.1 The aim of the experiment was to explore the role of C7 sugars (*D*-mannoheptulose, perseitol) and water supply on fruit softening from avocado fruit harvested in three different maturity stages defined by picking seasons (early-, mid- and late-season fruit).

1.2.2 Research objectives

The three main objectives of the study were:

1.2.2.1 To determine the amount of mesocarp water content and its effect on the softening pattern, if fruit are infused with water, and watery sugars.

1.2.2.2 To investigate, if pedicel infusion with C7 sugars, can maintain physiological concentrations of the C7 sugars *D*-mannoheptulose or perseitol in the mesocarp tissue harvested in three maturing season (early-, mid- and late-season fruit).

1.2.2.3 To investigate, if the continued supply of *D*-mannoheptulose or perseitol can delay the reduction in mesocarp deterioration and maintain fruit quality if fruit are infused in three maturing season (early-, mid- and late-season fruit).

1.3 LITERATURE REVIEW

1.3.1 The alteration of the sugar profile of avocado fruit from fertilization to fruit maturity in oil accumulating crops

Growth and development of avocado fruit has been researched intensively (Blakey *et al.,* 2009), but the softening process, a vital aspect of fruit ripening, is less well-investigated. In order to understand fruit softening, the understanding of early stage of fruit development and the associated physiochemical alterations is of paramount importance. Avocado fruit shows many unusual characteristics of physiological and morphological nature (Schroeder, 1953). One of the distinct features of avocado fruit is the large quantity of oil which accumulates in the mesocarp, while concomitantly the fruit water concentration, commonly termed the fruit water content, decreases (Bower and Cutting, 1988; Eaks, 1990).

Additionally, avocado fruit will not ripen, while attached to the tree; hence, they must be detached from the tree to be able to soften and to become edible (Jeong *et al.,* 2002). Avocado fruit growth follows a sigmoidal pattern from fruit-set to harvest maturity (Bower and Cutting, 1988), with the extend of fruit growth determined by successful pollination (Czerednik *et al.,* 2015), fertilization and embryo development (Lovatt, 1990). Fruit growth, in general, is a result of cell expansion and cell division, processes depending on the translocation of carbohydrates synthesized in various parts of the plant (Schroeder, 1953). Cell division continues throughout fruit development; early fruit size increase can be associated to the seed growth (Mougheith *et al.,* 1978). (Blumenfled and Gazit, 1971), reported that numerous cultivars of avocado fruit grown in Israel found with high auxin concentration in the seed, tend to have fast mesocarp growth, which suggested that auxin as growth hormone regulator influence sink strength of growing fruit. Avocado seed is enclosed by an actively dividing mesocarp tissue (Mougheith *et al.,* 1978; Kotze, 1979), this tissue accumulates carbohydrates and proteins (Mougheith and Abdel-Hamid, 1978), that is second stage of phase 3 (rapid increase in fruit size).

Sucrose and symplastic solutes (glucose and fructose) are commonly stored as the polymer starch and, in avocado, glucose and fructose are present in nearly equal concentration during the early stages of development of the fruit (Cowan *et al.,* 1998), but at the onset of fruit ripening sucrose increases which result in an increase of total soluble sugar content (Li *et al.,* 2017). Tesfay *et al.* (2012) reported that these common sugars consisting of carbon 6 units, a vast amount of C7 sugars, particularly *D*-mannoheptulose and its reduced polyol form, perseitol, are synthesized in the avocado seed and in various parts of the avocado fruit. The C7 sugar *D*-mannoheptulose has been postulated to be associated with fruit quality (Liu *et al.,* 1999; Liu *et al.,* 2002), which has led to the assumption that it might be the substrate of respiration (Cowan, 2004; Bower and Bertling, 2005; Bertling *et al.,* 2007). Glucose, as a common sugar, also plays an important role as a precursor of the major antioxidant, ascorbic acid, which is found within the avocado exocarp tissue (Tesfay *et al.,* 2010).

The growth and development of fruit of many tree species can be divided into three phases: (i) cell division, (ii) an expansion phase involving cell enlargement and water accumulation and (iii) the ripening stage (Luckwill, 1959). During fruit growth and development, texture and firmness are closely associated with the cell wall composition, which provides not only rigidity and strength, but also a certain cell turgor. Cell wall thickness and strength are the main contributors to firmness and are largely determined by genetic factors (Toivonen and Brummell, 2008). Cell size and cell number of fruit influence the structural dry matter by determining the number of cell walls. This number also plays a role in fruit hardening and in many other qualitative characteristics, such as juiciness and shelf life (Czerednik *et al.,* 2015). As the fruit develops, changes occur in the cell wall, results in cell enlargement and membrane along with the influx of sugars, water, organic acids, and other compounds. These dissociable compounds allow the creation of a certain turgor pressure, necessary for fruit cell expansion and for keeping the fruit firm as it expands (Génard *et al.,* 2007).

To reach maturity, the avocado fruit requires a long developmental period, taking six to twelve months or more from flowering to harvest (Scora *et al.,* 2002). For example, in some cool avocado growing areas, the 'Hass' cultivar takes 14-18 months from flowering to fruit maturity (Bergh, 1975). Cell division occurs in most fruit only during the early fruit growth period; however, in avocado cell division is not restricted to the early development stage but can continue to and even beyond the time when the fruit reaches picking maturity (Bower and Cutting, 1988). In avocado, cell division only stops after physical separation of the fruit from the tree (Schroeder, 1953), the time when water and nutrient flow into the fruit is withheld. Giovannoni (2001) postulated that the mesocarp softening process is only initiated after fruit growth has ceased. During ripening fruit become soft-textured due to rapid biochemical and structural changes which lead to the loss of fruit firmness and to textural quality changes (Payasi *et al.,* 2009). Fruit ripening is a complex developmental process that involves the alterations of sugars, volatile substances (aroma) and pigments (Giovannoni, 2001; Gupta *et al.*, 2013).

The latter fruit attributes which involves color, aroma and texture are the result of decline in turgor due to respiration, a loosening of cell walls and the disassembly of cellular structures that is responsible for intercellular adhesion and providing structural rigidity (Brummell, 2006). These cell structure breakdowns result in changes in texture, partly due to water loss from fruit and due to an increase in osmotic solutes in the intercellular space (Brummell, 2006; Toivonen and Brummell, 2008). There are extensive modifications of cell wall polysaccharides by ripening-related enzymes that are transported into the intercellular space from the symplast. These changes significantly affect the strength and structure of the wall, and ultimately bring about fruit softening as part of the ripening process (Toivonen and Brummell, 2008).

1.3.2 Accumulation of sugars (storage reserve) photosynthate in tree crops

The dry mass of tree crops constitutes over 65% carbohydrates, and the sugars produced in avocado are the first products of photosynthesis and can immediately be used in tissue sinks and other growing organs (Wolstenholme and Whiley, 1997; Cowan *et al.,* 1998). Tesfay (2009) revealed that the major transportable carbohydrate in avocado is the seven carbon sugar *D*-mannoheptulose (Tesfay, 2009), with perseitol being less commonly transported (Minchin *et al.,* 2012). Whereas (Cowan *et al.,* 1997), reported sucrose as sugar that is translocated out of the leaf. Vascular bundles permeate the fleshy mesocarp and sucrose enters the fruit through them, and coalesce distally from where sucrose is transported to the major zone of phloem unloading in developing avocado fruit (Moore - Gordon, 1997). During the early fruit development stages, soluble sugars, such as fructose and glucose, are only mass produced in the seedling (Tesfay *et al.,* 2012).

Tesfay *et al.* (2012) reported an increase in C7 sugar concentration in shoot and cotyledon, with decrease in starch reserves in the cotyledon 'in the absence of light' during germination, which suggested that the plant then switches over to the C7 metabolism. Therefore, in avocado the progression from juvenility to maturity is characterized by a conversion from C6 sugar to C7 sugar metabolism. Individual leaves on avocado trees show an alteration from being net sinks to being net sources of sugars (carbohydrates). The ability of individual leaves to supply carbohydrates to nearby sinks is of paramount importance for yield, fruit size and quality (Wolstenholme and Whiley, 1997). Downton *et al.* (1987) reported that in citrus the availability of carbohydrates limits fruit set. Avocado is an evergreen tree that is known for producing more carbohydrates among other evergreen trees such as citrus, as it has an ability of converting C6 sugars to C7 sugars (Chandler, 1958, Tesfay *et al.,* 2012). However, deciduous trees largely depend on stored carbohydrates for early growth in spring, whereas in evergreens, such as avocado, this dependence is partly reduced by over-wintering leaves (Whiley, 1994).

1.3.3 The seasonal carbohydrate cycle in avocado and its relationship to tree phenology

Avocado seeds contain starch as a storage carbohydrate; this carbohydrate represent currently unutilized, but stored C6 sugar components (Liu *et al.,* 2002); while seedlings contains C7 sugars mannoheptulose and perseitol (Tesfay *et al.,* 2010). During early endosperm and embryo development, carbohydrates are required in vast amount for growth activity, until fruit reach maturity (Kozlowski, 1992), but avocado appears to accumulate high carbohydrate reserves level that are manufactured during photosynthesis in the leaves as means of adaptation to drought and water stress (Whiley and Wolstenholme, 1997). Carbohydrate reserves are accumulated before harvest, and form a reserve which is eventually used as energy source for the respiratory process postharvest (Kozlowski, 1992), but reserves are easily depleted during fruit growth, particularly when there is limited assimilates that are produced during photosynthesis (Whiley and Wolstenholme, 1990).

One of the major factors that have often been suggested to restrict fruit production are inter- and intra-seasonal competition for limited carbohydrate resources (Whiley and Wolstenholme, 1990; Schaffer *et al.* 2013). Whiley and Wolstenholme (1997) suggested that the level of starch reserve at critical periods can be used in orchard management decisions as they tend to follow a seasonal pattern, peaking just before flowering, decreasing rapidly during flowering and fruit set, starch remaining low until mid-summer, and increasing through autumn and winter. In avocado, the summer growth flush is the main contributor to starch accumulation, but if summer flushing is extended in the trees, starch accumulation can be delayed which may ultimately affect the potential starch levels for that season (Whiley and Wolstenholme, 1990).

Whiley and Wolstenholme (1989) proposed a quantitative index as a management tool, which integrates the phenological growth model with a starch curve as an orchard management tool. It mainly involves phenological events that directly contribute to the carbohydrates reserve

concentrations in the leaves and critical periods where competitive sinks have limited sources that are produced during photosynthesis. The phenological cycle depicts that, carbohydrates (starch) reserves are at a peak when growth demands are lowest, during the prolonged winter rest period. During flowering in winter and fruit set starch is required in vast amounts, as a result, starch reserves drop rapidly and reach the lowest concentration in the summer fruit drop period (Whiley and Wolstenholme, 1990) (Figure 1). Whiley and Wolstenholme (1997) reported that greater root growth in avocado contributes to high starch levels which concur with Whiley (1994) and Whiley *et al.* (1996) report on crop failure that was more associated with poor flowering, possibly due to inadequate root growth during floral induction.

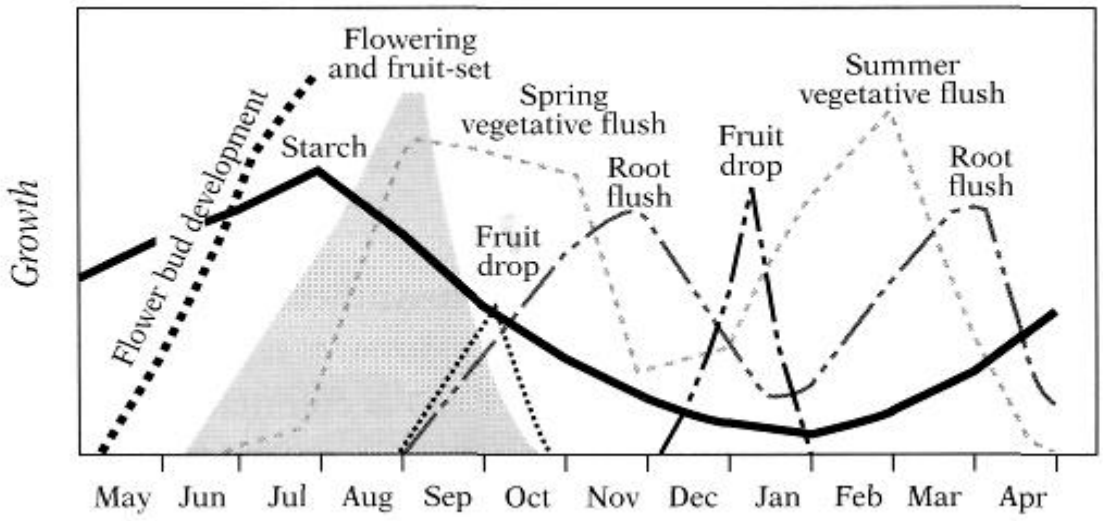

Figure 1: The growth cycle of avocado, cv Fuerte, with relationships between vegetative and reproductive growth and reserve starch in the trunk of trees (Whiley and Wolstenholme, 1990).

1.3.4 Polyols (sugar alcohols) as storage compounds in higher plants

Polyols also known as sugar alcohol are the reduced form of aldose and ketose sugars (and their derivative) to form a straight (acyclic polyols or alditols) or branched chain polyols (cyclic polyols or alditols) (Noiraud *et al.*, 2001). The term polyols refers to compounds consisting of three or more carbons bearing each of them bearing hydroxyl group (Da Costa *et al.*, 1998).

Polyols are produced by plants through photosynthesis, and they undergo direct oxidation to sugars, but before oxidation to a sugar phosphate, the preliminary phosphorylation of the polyol may also take place (Lewis and Smith, 1967). Plant metabolism is characterized by a major role played by variety of other sugars such as stachyose in cucurbits (Pharr *et al.*, 1985; Taji *et al.*, 2002). Some are polyols (sugar alcohols), such as perseitol in avocado (Shaw *et al.*, 1980), inositol in grains (Loewus and Loewus, 1983), mannitol in apple (Makinen and Soderling, 1980), and sorbitol in peach (Makinen and Soderling, 1980). In addition to their active involvement in photosynthesis and translocation, similar to sucrose but these partitioning differences, reveal that polyols also serve as storage compounds (Tesfay *et al.*, 2011), which make sugar alcohol to have chemical advantage over C6 sugar (sucrose) as energy stores (Lewis, 1984).

However, perseitol is the major polyol in avocado seed, and could be transported from the seed into the developing seedling (Tesfay *et al.*, 2012). Tesfay *et al.*, 2012 reported that *D*-mannoheptulose was present in trace amounts in the seed, which suggest that *D*-mannoheptulose was released from its reduced form perseitol. Sugar alcohol perseitol and *D*-mannoheptulose have been reported to be in all major tissues of avocado plant (Liu *et al.* 1999), showing a key function of these compounds in avocado growth and development. In addition to functions for the C7 sugars existing in plants are similar to known functions of C6 sugars (Zimmermann and Ziegler 1975; Nadwodnik and Lohaus 2008) include carbohydrate transport as a transport form for reduced carbon (Noiraud *et al.*, 2001; Liu *et al.*, 2002). Polyols have been also shown to serve as carbohydrate reserves (Oliveira and Priestley 1988) as compatible solute synthesized in response to abiotic or biotic stress (Loescher, 1987), or as osmoprotectants (Morgan 1984).

Sugars plays an important role not only in growth and development of avocado fruit, but are also considered to be important during fruit ripening as respiratory substrates (Liu *et al.*, 2002). Tesfay (2010) demonstrated the multifunctional roles of avocado carbohydrates as 'sources of energy', storage and phloem-mobile transport sugars, and *D*-mannoheptulose as a major fruit antioxidant. It is thought that carbohydrate allocation within a tree determines vegetative growth, annual fruit set, fruitlet abscission, and fruit growth (Cowan *et al.*, 1997). Tesfay *et al.* (2010) reported vast amounts of the C7 sugars *D*-mannoheptulose and its reduced form, perseitol, in all tissues of the avocado fruit. The high concentration of C7 sugars in avocado fruit has led to suggestions that they control ripening (Liu *et al.*, 2002) or may be associated with fruit quality (Bertling and Bower, 2005).

It has also been proposed that the differences in sugar content between the cultivars and growing regions could affect the postharvest fruit quality (Foyer and Noctor, 2005; Kaluwa, 2010). Donetti and Terry (2014) reported that C7 sugars decrease as fruit soften. In avocado fruit, the accumulation of oil is a prominent attribute of fruit maturity, the formation of the fats depends on the hydrolysis of the carbohydrates to acetate with the subsequent synthesis of fatty acids from this acetate. Glycerol is another sugar metabolism product, which combines with fatty acids to form fats (Bean, 1958). It has been suggested in various studies that changes in carbohydrate (sugar) reserves as well as fruit quality may be directly affected by seasonal growth and time of harvest (Whiley and Wolstenholme, 1990; Kaiser and Wolstenholme, 1993), therefore the correct time of harvest and maturity stage is of paramount importance.

1.3.5 The role of cell wall degrading enzymes in fruit softening

Ripening of avocado is the result of cell wall integrity degradation, resulting in the loss of cell to cell cohesion (Platt-Aloia and Thomson, 1981). Avocado cell walls consist of cellulose, hemicellulose, and pectin (Goulao and Oliveira, 2008; Scott *et al.*, 1963). Ripening of avocados

occur at the same time with softening of the fleshy mesocarp tissue of the fruit, which is believed to be caused by the loss of cell to cell cohesion with the cell walls (Platt-Aloia *et al.*, 1981). Scott *et al.* (1963) found that the major component of avocado cell walls is cellulose. Pesis *et al.* (1978) reported that during fruit softening cellulase tend to increase, which is the process associated with ethylene production peak and high CO_2 production. The same authors highlighted an increase in cellulase activity, when avocado fruit was placed in an ethylene-rich environment. During ripening in avocados, pectinmethylesterase (PME) activity decreases, while cellulase and polygalacturonase (PG) activities have been found to increase (Awad and Young, 1979). Subsequently, Hatfield and Nevins (1986) identified the enzyme (1-4)-β-D-glucanase by purifying avocado cellulase. These authors also found that (1-4)-β-glycosyl linkages is hydrolysed by (1-4)-D-glucanase only and not the cellulose polymers found in mature avocados, which meant that the breakdown of avocado cell walls could not be solely responsibility of cellulase activity.

Hatfield and Nevins (1986) suggested that cellulose fibrils are also hydrolysed, and the consistent observations of changes in cellulose fibres, can be seen under microscope. PG activity increases after cellulase activity first increases (Awad and Young, 1979), and this phenomenon may be due to alteration of hydrogen bonding to other polysaccharides in the cell wall, thus disturbing the cell wall matrix and allowing enzymes to break down the polygalacturans (Hatfield and Nevins, 1986). The strong correlation between softening and cellulase activity suggest that cellulase is the enzyme responsible for the initial stages of fruit softening (Hatfield and Nevins, 1986). This is controlled in part by ethylene, while PG seems to be responsible for final fruit softening (Bower and Cutting, 1988).

1.4 DISCUSSION AND CONCLUSION

In avocado, as in any fruit tree, fruitlet abscission, vegetative growth, annual fruit set, and fruit production are determined by the sugar allocation within the avocado tree (Liu *et al.*, 2002). These C7 sugars, particularly *D*-mannoheptulose and its reduced polyol form, perseitol, are not only storage compounds in mesocarp tissue, but *D*-mannoheptulose could also play a vital role as antioxidants (Tesfay *et al.*, 2010). Further work on C7 sugars has indicated that these compounds may provide protection against stress (Liu *et al.*, 2002). It has also been found that a further uncommon C7 sugar alcohol, volemitol, plays a vital role in carbon translocation and storage, assimilation, provision of reducing power, and protection against various stresses in certain species, such as *Primula* (Häfliger *et al.*, 1999); volemitol is also found in avocado fruit (Cowan, 2004). The putative role played by C7 sugars in ripening and their known antioxidant activity have led to this study of the role of C7 sugars in postharvest fruit softening of avocado.

Liu *et al.* (2002) suggested that C7 sugars could control softening of the fruit by controlling the ripening process, acting as ripening inhibitors when still attached to the tree, only allowing for the triggering of softening when sugar levels declined after detachment from the tree. Further work on C7 sugars has indicated that C7 sugars may provide protection against stresses due to their reducing power (Liu *et al.*, 2002). Bertling *et al.* (2007) proposed that the decline in C7 sugars, as the fruit nears harvest maturity, could be related to deterioration in post-harvest quality, ultimately affecting the softening process. It has been found that a sugar alcohol, Volemitol, plays a pivotal role in carbon translocation, storage and assimilation, and protection against various stresses in a certain Primula species (Hafliger *et al.*, 1999), and this sugar alcohol has reportedly been found within avocado fruit as well (Cowan, 2004). Ogata *et al.* (1972) studied the C7 sugars content particularly mannoheptulose of four different avocado cultivars and reported that unripe mesocarp tissue contained relatively high levels (0.64-2.5%),

while ripe mesocarp tissue contained lower levels (0.03-0.5%), which suggest that a decrease in C7 sugars eventually triggers softening in avocado fruit. Perseitol is a polyol (sugar alcohol) that is known as a storage compound in avocado (Tesfay *et al.*, 2012), and polyols is also known to act osmotically, balancing osmotic pressure differences from inside the cell to outside, function as compatible solutes and causing water influx. Such solutes replace water molecules by means of their water-like -OH groups and thus participate in the water-enforced hydrophobic interactions so critical to biological activity. The equivalent of complete hydration of biological polymers is therefore maintained, even with a reduced number of available water molecules (Yancey *et al.*, 1982). (Faraji and Lindsay, 2004), have found that certain C6 sugar alcohols (sorbitol and mannitol) can act as antioxidants. In the same way, C7 sugar could form an important part of the pool of antioxidants in avocado, protecting the fruit from oxidative stress, mesocarp deterioration and, certain role in softening process. Therefore, by infusion of watery sugars such as mannoheptulose, perseitol and sucrose in the pedicel we are simulating the attachment of the fruit from the tree through maintaining a continuous supply of carbon sugars with water within the fruit, and investigate their effect in fruit quality.

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CHAPTER 2

EFEECTS OF SEVEN CARBON SUGAR INFUSION ON THE RIPENING PATTERN OF 'HASS' AND 'FUERTE' AVOCADO (*Persea americana* Mill.) FRUIT FROM DIFFERENT HARVESTING SEASONS

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ABSTRACT

A clear understanding of avocado (*Persea americana* Mill.) postharvest physiology is required for improved management of this crop. Avocado fruit is very susceptible to a large variety of disorders, and these disorders maybe the result of a lack of stress resistance in the tissue involved. The C7 sugars *D*-mannoheptulose and perseitol have been reported to impart antioxidant activity to avocado mesocarp tissue and seem, therefore, associated with avocado fruit quality. In order to investigate, if the concentration of the prominent C7 sugars, *D*-mannoheptulose and perseitol, in the mesocarp can be maintained through infusion of these sugars, sugar solutions were infused into commercially mature, non-ripe avocado fruit through the fruit pedicel. As controls fruit were either not infused, infused with water or infused with 9.5 mM/ 4.75 mM sucrose. Fruit firmness, respiration (CO_2 production), and fruit internal and external quality as well as shelf life were determined over the postharvest ripening period. The infusion of the C7 sugars *D*-mannoheptulose and perseitol, tended to maintain the fruit firmness during the postharvest infusion period in fruit infused with these sugars. It was also noted that the C7 sugar treatments 9.5 mM *D*-mannoheptulose, 4.75 mM *D*-mannoheptulose, 9.5 mM perseitol and 4.75 mM perseitol had the lowest of CO_2 production; however, the results showed that there was a significant difference between infusion treatment means in all harvesting seasons for both, 'Fuerte' and 'Hass' avocados. Firmness of early-harvested fruit tended to delay and decrease significantly after 9 days of postharvest infusion, particularly in 'Hass'. Moreover, it was noted that during the in early-season some 'Hass' fruit turned rubbery, while 'Fuerte' fruit shrank considerably; some early-season fruit never reached fully ripeness. The differences between infusion treatments remained significant, even twelve days after postharvest days. Similarly, fruit infused with 9.5 mM *D*-mannoheptulose, 4.75 mM *D*-mannoheptulose, 9.5 mM perseitol and 4.75 mM perseitol were the firmest throughout the

harvesting seasons, followed by water-infused fruit. Sucrose infusion had a least effect on maintaining fruit firmness, and there was no significant difference between water-infused fruit and sucrose-infused ones. Firmness of sucrose-infused fruit tended to decrease significantly after day 9 of infusion. Late-season fruit took a shorter time to ripen than early- and mid-season fruit. *D*-mannoheptulose (4.75 mM and 9.5 mM) as well as perseitol (9.5 mM and 4.75 mM) infused fruit had a significantly reduced respiration, maintaining the lowest CO_2 production during all three harvesting seasons. Postharvest disorders (anthracnose and vascular browning) were significantly affected by postharvest treatment and the interaction of harvesting season (early-, mid- and late-) of 'Hass' and 'Fuerte'. In the early and late-harvest season, control fruit had highest postharvest disorder infections, particularly vascular browning, followed by sucrose-infused fruit. The high levels of anthracnose incidences were recorded in 4.75 mM sucrose infused fruit from the mid-harvest season. Fruit infused with *D*-mannoheptulose and perseitol had no anthracnose incidences and no vascular browning, as well as water-infused fruit tends to follow the same pattern. However, vascular browning and anthracnose was observed in 'Fuerte' and 'Hass' from the late-harvest season of water-infused fruit. It is, therefore, suggested that a decrease of *D*-mannoheptulose and perseitol concentration to a certain level in the mesocarp at postharvest triggers ripening, while postharvest water-loss and the aligned decrease in firmness concomitantly occurs. The C7 sugars *D*-mannoheptulose and perseitol play various important roles in avocado; therefore, achieving and maintaining a certain level of these uncommon sugars in the mesocarp pre-and-postharvest is a vital factor in ensuring and maintaining good fruit quality.

Keywords: *C7 sugar infusion, 'Hass', 'Fuerte', D-mannoheptulose, perseitol, sucrose, early-harvest, mid-harvest, late-harvest, harvesting season, CO_2 production, respiration, firmness anthracnose, vascular browning*

2.1 INTRODUCTION

The production of avocado (*Persea americana* Mill.) has been increasing rapidly worldwide, so that the commodity is amongst the most-commonly sold subtropical fruit in the world, with an estimated annual production of more than 4.7 million tonnes (FAOSTAT 2015). Avocado is often consumed due to its distinct attributes as a highly nutritious fruit, containing high amounts of thiamin, Vitamin E and C, while also being rich in manganese, phosphorus and iron (Naveh *et al.,* 2002). The shelf life of avocado is, however, limited due to a very high postharvest fruit respiration rate. Fruit ripening is the initial phase of fruit senescence, which is characterized with changes in the membrane and composition of the avocado fruit mesocarp (Bower and Cutting, 1988; Van Rooyen and Bower, 2005). During avocado ripening, there is a loss in cell compartmentation, which activates membrane-degrading enzymes, resulting in increased membrane permeability (Ahmed *et al.,* 2010; Platt-Aloia and Thomson, 1981; Van Rooyen and Bower, 2005). Huber *et al.* (2001) define the ripening process 'as a form of programmed organ death which leads to a decline in fruit firmness' due to an increase in activity of polygalacturonase (PG), a burst in ethylene production and signal transduction (Bleecker and Kende, 2000; Hershkovitz *et al.*, 2005), and softening (Wakabayashi and Huber, 2001). Fruit softening is a vital part of the ripening process that is determined by changes in the cell wall (Brummell and Harpster, 2001), followed by a high rate of water loss due to the high respiration rate, which decreases the time the fruit takes to ripen postharvest (Lallu *et al.*, 2004). Ripening as a later developmental stage of the fruit affects several quality factors, such as flavour, firmness, colour, shape and texture of the fruit (Cai *et al.,* 2006). The postharvest ripening rate is associated with fruit maturity, as fruit harvested at the early harvest of the season take longer to get ripen than fruit harvested at late season (Adato and Gazit, 1974). During ripening initiation, multiple anabolic changes demanding high-energy input occur, resulting in a rapid increase in respiratory activity. Liu et al. 1999 proposed that C7 sugars

play various important roles in avocado fruit development, with *D*-mannoheptulose as the source of energy, while Tesfay *et al.* (2010) found that this sugar alcohol serves as an anti-oxidant in the avocado mesocarp and perseitol is a storage compound (Tesfay *et al.*, 2012). The potential involvement of these C7 sugars in inhibiting or triggering fruit ripening, once the fruit is physically separated from the tree, remains unclear. Softening of avocado fruit can only occur, once the fruit is removed from the tree (Jeong *et al.*, 2002); however, certain pre-harvest factors may result in a variable ripening, which is aligned with uneven softening, creating substantial logistical problems, especially when fruit are ripened in pre-packaging facilities (Bower *et al.*, 2003). An increase in water stress during postharvest, for example, also reduces the normal time to ripen and may lead to high postharvest disorder incidence, particularly of anthracnose and mesocarp discoloration (Bower and Cutting, 1988). The cause of ripening variation amongst fruit of the same size and origin is still unknown, and flowering period which cause a wide variability in fruit age at harvest does not fully explain uneven ripening of avocado fruit.

2.1.1 Research aim

2.1.1.1 The aim of this experiment was to explore the role of C7 sugars (*D*-mannoheptulose, perseitol) and the common C6 sugar sucrose, as well as maintenance of the water supply to the avocado fruit on the softening pattern during three maturity stages as defined by picking seasons (early-, mid-, and late-season fruit).

2.1.2 Research objectives

2.1.2.1 To enhance the postharvest shelf life of ‘Fuerte’ and ‘Hass’ avocado fruit through continuous infusion of C7 sugars, sucrose or water.

2.1.2.2 To investigate, if the continued supply of sugars (*D*-mannoheptulose, perseitol, sucrose) and water can delay the mesocarp deterioration and maintain fruit quality.

2.1.2.3 Comparing the effects of such sugar (*D*-mannoheptulose, perseitol, sucrose) infusion on the ripening pattern of 'Fuerte' and 'Hass' avocado fruit.

2.2 MATERIALS AND METHODS

2.2.1 Fruit material and experimental design

During the 2015 and 2016 avocado growing season fruit were collected from 'Hass' and 'Fuerte' avocado orchards from Bounty Farm, Winterskloof, in the KwaZulu-Natal Midlands, South Africa (29°28´S; 30°161´E). Avocado fruit were harvested with pedicels and fruit selection was based on uniformity of shape, colour and size. Only bruise- and blemish-free fruit were used in the experiment. To prevent physical damage, harvested fruit were gently placed into crates and immediately transported, in a well-aerated vehicle, to the Horticultural Science laboratories at the University of KwaZulu-Natal, Pietermaritzburg. Three (3) separate experiments were set up throughout 2015 and 2016, one experiment for each harvesting seasons. Avocado fruit within a mass range of 203-243 g (class 1; Government Gazette 37223, 17 Jan 2014) were harvested during three seasons: 'early' (29/04/2016; moisture content 72%, equivalent to 28 % DM and 25% oil), 'mid' (09/09/2015; moisture content 66%, equivalent to 34% DM and 26 % oil) and 'late' (01/12/2015; moisture 60% moisture, equivalent to 40% DM and 31 % oil). For each experiment 96 avocados with 5 cm pedicels were acquired, with 48 'Hass' and 48 'Fuerte' fruit. Fruit were kept at room temperature and randomly divided into nine treatments: seven infusion treatments and two controls (fruit with pedicel and fruit without pedicel). In each experiment, fruit were assessed for firmness and CO_2 production throughout their shelf-life, being measured in three (3) day intervals of the postharvest storage period (day 0, 3, 6, 9, 12, and 15).

2.2.2 Treatments

Silicon tubing of 5 mm length was attached to the pedicel (retained after harvest and re-cut prior to treatment to remove any dead tissue) of treated fruit, and sealed with petroleum jelly at the base (Bower and Cutting, 1987). According to Bertling *et al.* (2011), an infusion of 1.5 mL water, sucrose (C 6): 1.5 mL of 9.5 mM/fruit; 4.75 mM/fruit solution and C7 sugars solutions 1.5 mL of 9.5 mM/fruit; 4.75 mM/fruit *D*-Mannoheptulose, 9.5 mM/fruit; 4.75 mM/fruit Perseitol solution) was infused to 6 fruit per treatment. Control (fruit with pedicel and without pedicel) fruit were untreated.

2.2.3 Postharvest infusion data

External, visual observations were made before and after postharvest infusion and fruit firmness, CO_2 evolution, and overall fruit condition were measured and assessed. Fruit were visually rated for shrivelling, and diseases infection. After 3 days interval of postharvest infusion, fruit firmness, CO_2 evolution, postharvest disorder was calculated. Postharvest infusion data was collected in 3 days interval until day 15 of postharvest.

2.2.4 Measurement of fruit firmness

Firmness of each fruit was measured from day of infusion and in three days after infusion, until ripening was reached using a hand-held firmness tester (5 mm anvil; Densimeter, Bareiss, Oberdischingen, Germany). Fruit firmness, fresh weight loss, respiration was monitored according to Tesfay et al. (2010) and Tesfay et al. (2011). The same fruit was measured each day, taking two measurements along the equatorial region of the fruit. Fruit was deemed to be ‘ripe’ when softness measurement reached 6N (Köhne *et al.*, 1998).

2.2.5 Measurement of respiration rate

Fruit respiration rate, as determined by CO_2 production, was measured using an infrared gas analyser (EGM-1, PP Systems, Hitchin, Hertfordshire, UK) with an error of less than 1% of span concentration over the calibrated range. Treated avocado fruit were incubated in 1 litre plastic jars for 15 minutes. Net CO_2 production per kilogram fruit was calculated by adjusting for headspace, ambient CO_2 in the jar, fruit volume and fruit mass. Carbon dioxide concentration (µl L^{-1}) was determined daily and results calculated as rate of CO_2 production per hour (ml kg^{-1} FM h^{-1}).

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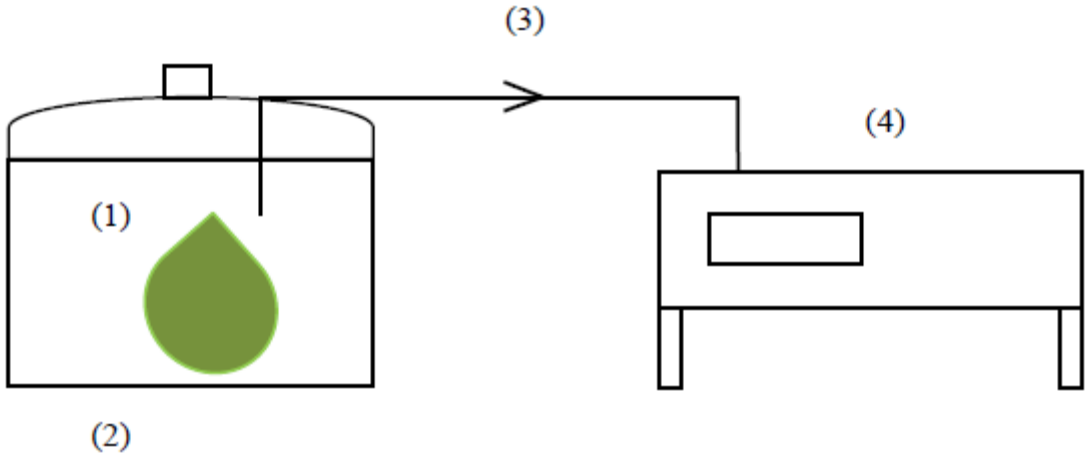

Figure 2: Carbon dioxide determination: (1) avocado; (2) 1000 ml glass jar containing the sample; (3) duct conveying gas into the EGM-4 Environmental Gas Analyzer; (4) EGM-4 Environmental Gas Analyzer (Kassim *et al.*, 2013).

The amount of carbon dioxide (ml· kg^{-1} FM · h^{-1}) released was calculated using the following equation:

$$CO_2 = \frac{Net\ CO_2}{1000} \times Headspace \times \frac{1000}{m} \times \frac{60}{t} \qquad (3.1)$$

Where:

CO2 = carbon dioxide released from avocado fruit

Net CO2 = fruit CO2 – ambient CO2 [ml],

Headspace = container volume – fruit volume [l],

m = fruit mass [g], and

t = time of incubation.

2.2.6 Internal Quality Assessment

Over the ripening period, all six replications per treatment (six fruit) were cut into halves and assessed for anthracnose and vascular browning. Internal assessment was made on a scale of 0 (no visible symptoms) to 5 (extremely severe, area completely infected or discoloured). Fruit that ripened to the eating ripe stage with a rating of 0 for all internal disorders and body rots (*i.e*., free from any disorders and diseases).

2.2.7 External Quality Assessment

The severity of external disorder was rated similarly on a scale of 0 (no blemishes) to 10 (fruit surface area entirely blemished). The fruit were visually assessed for external disorder from day 0 of postharvest infusion until fruit reach edible ripening.

2.2.8 Statistical Analysis

The data were analyzed in each treatment combination consisted of six fruit, each fruit constituting a single replication. Analyses of variance was performed using GENSTAT (edition 17th; VSN International, Hemel Hempstead, UK). Standard deviations (SD) were calculated and differences among treatments were separated by a significant difference (LSD) test at $p \leq 0.05$.

2.3 RESULTS

2.3.1 Firmness

Fruit firmness differed significantly between treatments and over the postharvest infusion period for all harvesting seasons for both, 'Fuerte' and 'Hass' avocados. As expected, fruit firmness decreased with an increase in postharvest storage days. Harvesting seasons also had a significant effect in fruit firmness ($p \leq 0.05$), with mid-, and late-harvest fruit firmness being significantly higher than in early-harvest fruit. Particularly fruit infused with 9.5 mM *D*-mannoheptulose I, with 12, 056 N as difference between two mean values and 9.5 mM perseitol I, with 11, 074 N as difference between two mean values at LSD ($p \leq 0.05$) = 6.447 (Figure 2). From the early harvest showed a tendency towards delayed softening and a significant decrease after 9 days of postharvest infusion; this was particularly visible in 'Hass' fruit (Figure 1). It was also noted that some early-season 'Fuerte' fruit turned rubbery, while in 'Hass' fruit shriveling was noticed, and some early-season fruit never reached full ripeness (Figure A1 C&D). In generally, it was observed that fruit harvested during the early season showed more uneven ripening than mid- and late season fruit. Fruit firmness decreased, however, significantly after 6d of postharvest infusion in fruit from the mid-, and late-harvesting seasons (Fig 3- 6). Fruit with the pedicel attached retained firmness better than fruit without pedicels but there was no significant difference regarding firmness particularly in late-season 'Fuerte' fruit with 7.968 N as difference between mean values in pedicel attached fruit and 5.967 N as difference between mean values in fruit without pedicel, with LSD ($p \leq 0.05$) = 10.485 (Fig 4). The differences between infusion treatments remained significant, even on day twelve of postharvest days. Similarly, C7 sugar (9.5 mM *D*-mannoheptulose I, 4.75 mM *D*-mannoheptulose II, 9.5 mM perseitol I and 4.75 mM perseitol II) -infused fruit were the firmest throughout the harvesting seasons, followed by water infused fruit. Both sucrose (9.5 mM and 4.75 mM) infusions retained the least fruit firmness, lower than the controls, in all harvesting

season for both cultivars. Water infusion retained firmness and reduced the ripening heterogeneity in mid- and late-season fruit. Late season fruit took a shorter time to ripen than early and mid- season ones (Fig 2- 6). According to Blakey *et al.* (2009), water infusion through the pedicel decreases the ripening heterogeneity in mid- and late-season fruit. The present findings also concur with the observation, that early-season fruit have a lower water content, resulting in faster fruit ripening, possibly due to water stress and higher fruit ABA biosynthesis that stimulates ethylene production, which triggers ripening (Bower and Cutting, 1988).

2.3.2 Respiration rate (CO_2 production)

Fruit infused with carbon-7 sugars (9.5 mM of *D*-mannoheptulose, 4.75 mM of *D*-mannoheptulose, 9.5 mM perseitol and 4.75 mM perseitol) had a significantly reduced CO_2 production ($p \leq 0.05$), maintaining the lowest CO_2 production in all three harvesting seasons (Fig 7- 12). In all harvesting seasons, the respiration rate of control fruit was higher than water infused fruit. There was significance difference in control fruit and sucrose- infused fruit from day 0 to day 6 of postharvest infusion, with 9.242 $ml \cdot kg^{-1}$ $FM \cdot h^{-1}$ as difference between mean values in pedicel attached fruit and 8.924 $ml \cdot kg^{-1}$ $FM \cdot h^{-1}$ as difference between mean values in sucrose- infused fruit, with LSD ($p \leq 0.05$) = 11.848 (Fig 8). After 6 days of postharvest infusion, control fruit and sucrose-infused fruit showed an increased respiration in mid-, and late-harvest season fruit, reaching a peak on day 9 of the postharvest infusion (Fig 9- 12); sucrose infusion, on the other hand, showed no effect on maintaining fruit quality. Water infusion had a considerable effect on mid- and late harvested fruit (Fig 9- 12), but not on early-harvested fruit. The CO_2 production of fruit increased during the postharvest observation period, peaking on day 6; thereafter a decreasing trend was noted until fruit were fully ripe. The CO_2 production of both 'Hass' and 'Fuerte' was lower in C7 sugar (*D*-mannoheptulose and perseitol) -infused fruit, during mid-harvest, respiration decreased significantly towards

the end of the observation period. In the mid-harvest, sucrose-infused fruit, however, showed a similar trend in respiration as water-infused fruit, reaching a respiratory peak after 6 days of postharvest infusion (Fig 9- 10).

2.3.3 Internal and External Quality Assessment

The two major postharvest problems detected (anthracnose and vascular browning), were significantly ($p \leq 0.05$) affected by the interaction of postharvest infusion and harvesting season (early, -mid; and late-harvest) of 'Hass' and 'Fuerte'. Results showed that, in early and late-harvest season, control fruit of both cultivars had the occurrence of vascular browning (Figure 1A), followed by sucrose-infused fruit (Fig 13- 14). Lower levels of anthracnose incidence were recorded in 4.75 mM sucrose-infused fruit of mid-harvest season (Fig 16), than control fruit (fruit with pedicel and fruit without pedicel) of Fuerte late-harvest season (Fig 15). Fruit infused with *D*-mannoheptulose and perseitol had no anthracnose incidence and vascular browning; water-infused fruit tended to show low postharvest problems (Fig 13-15). High incidences of vascular browning and anthracnose were however, observed in 'Fuerte' and 'Hass' during the late-harvest season (Fig 13- 16).

2.4 DISCUSSION

As in any climacteric fruit, the ripening behavior of avocado is characterized by a rise in respiration rate during the onset of ripening followed a quick decline (Millerd *et al.*, 1962; Liu *et al.,* 1999). In avocado, the ripening process is aligned with fruit softening; hence, firmness readings of avocado fruit are indicative of both, its maturity and the stage of postharvest ripening (Peleg *et al.,* 1990). During growth and development of avocado, C7 sugars are stored; however, immediately after harvest these sugars are utilized for postharvest physiological process, such as respiration, through enzymatic activity that metabolizes these C7 sugars (Kader and Yahia, 2011). The present study concurs with these findings, as there was a

significant increase in CO_2 production after nine days of fruit infused with 9.5 mM sucrose, 4.75 mM sucrose and control (fruit with pedicel and without pedicel) (Figure 7 -12). The rise in CO_2 production after nine days may also indicate the reduction in mesocarp C7 sugars. Avocado is a highly perishable agricultural commodity, with a short shelf-life due to its high respiration rate (Saltveit, 1996); the fruit is unique in that the main respiratory substrates are C7 sugars instead of C6 sugars (Liu *et al.,* 1999; Bower and Bertling, 2005; Meyer and Terry 2008). The respiration over time of avocado fruit is inversely related to shelf-life, because respiration directly relates to the breakdown in quality parameters, e.g. sugar content, flavor and aroma compounds and firmness (Kaluwa, 2010). A high CO_2 evolution indicates a high respiration rate; therefore, fruit are deteriorating quickly and, therefore, fruit quality decreases (Wills *et al.,* 1989; Kaluwa, 2010). Fruit infused with *D*-mannoheptulose and perseitol were much firmer and had a lower respiration rate than non-infused, water- infused and sucrose infused fruit of 'Hass' that was harvested mid-season (Fig 1-12). Fruit infused with this C7 sugars, in mid-harvest were firmer than late-harvested fruit, which suggests that *D*-mannoheptulose could be the 'ripening inhibiting factor', concurring with findings by Liu *et al.* (1999) that there was high amount of C7 sugars in the mesocarp, which act as antioxidant during ripening. In mid-season firmness of mid-harvest 'Fuerte' decreased significantly faster than that of fruit from other season, which may indicate that in September, 'Fuerte' was already at the ripening stage at the mid-harvest picking, but not at the early harvest, as previously suggested (Adato and Gazit, 1974; Cowan and Bornman, 2004). This suggests that *D*-mannoheptulose could be the elusive 'ripening inhibiting factor' (Tesfay *et al.,* 2012). The normal plant cell metabolism is maintained through utilization of energy compounds, the rate of CO_2 production is an estimate of the amount of energy used (Nilsen and Orcutt, 1996). The fast water loss, in early-harvested fruit (Fig 1-2), compared with mid-harvest fruit (Figure 3-4), which then resulted in shriveling of some fruit and an increase in postharvest disorder

incidences (Figure 13-15), such as mesocarp discoloration and anthracnose; these results coincide with previous reports (Bower and Cutting, 1988). The fruit physical changes during ripening are also related to fruit maturity, as early-season tended to shrivel (Figure A1D) and takes longer time to get ripen, unlike fruit harvested mid- season (Zauberman and Schiffmann-Nadel, 1972). Maintaining the presence of *D*-mannoheptulose and its reduced form, perseitol seemed to have slowed down the rate of respiration in late-harvested fruit (Figure 11-12). Fruit maturity at harvest is an important factor determining storage-life and the final fruit quality (Kader, 1999). Late-season fruit took a shorter time to ripen than early- and mid-season fruit (Figure 5-6); this may be result of the more advanced fruit development, as fruit hung longer on the tree (Cutting and Wolstenholme, 1992). This phenomenon of quicker ripening of late-harvested fruit is a result of dramatic increase in rate of respiration, and also accelerated by ethylene synthesis (Paul and Pandey, 2014) resulting in CO_2 release and faster ripening. These results concur with Munzhedzi (2016), in that harvesting-season could affect the ripening of avocado fruit. Increasing the time of exposure to an inoculum, increases the rate of infection (Saranada *et al.*, 2004); hence, in fruit from the late season, control, water- and sucrose-infused fruit had a high incidence of postharvest infections due to the increased time of exposure to inoculum, while hanging on the trees. Bower and Magwaza (2004) suggested that late-season fruit could also be more prone to postharvest disorder, such as chilling injury, due to the low moisture content at time of fruit harvest. The high rate of water loss after harvest increases the incidence of rots by 5 – 15 % (Bower and Cutting, 1988; Lallu *et al.*, 2004). Postharvest infusions with *D*-mannoheptulose and perseitol seemed to be most beneficial in suppressing respiration. Comparing untreated fruit, with those remaining pedicel tended to maintain better fruit quality, concurring with Landahl *et al.* (2009), who found higher amounts of *D*-mannoheptulose near the pedicel of the fruit.

2.5 CONCLUSION

The heterogeneity of avocado ripening is a result of the fruit's intricate physiology, pre-harvest, and postharvest factors. Avocado does not ripen while hanging on the tree; this phenomenon allows fruit of very different ages to hang in close vicinity in a tree, making it difficult to predict postharvest ripening. The findings of this study show that postharvest quality parameters of 'Hass' and 'Fuerte' avocado are significantly affected by the continuous supply of C7 sugars and water. As continuous infusion of *D*-mannoheptulose, and its reduce polyol form perseitol, reduces fruit respiration and retains firmness of avocado fruit, these findings confirm earlier assumption by Bertling and Bower (2006) that the C7 sugars found predominately in the mesocarp tissue play an important role in avocado fruit quality. Infusion of C7 sugars as postharvest treatment, seem to contribute severely to the synchronization of avocado ripening.

FIGURES

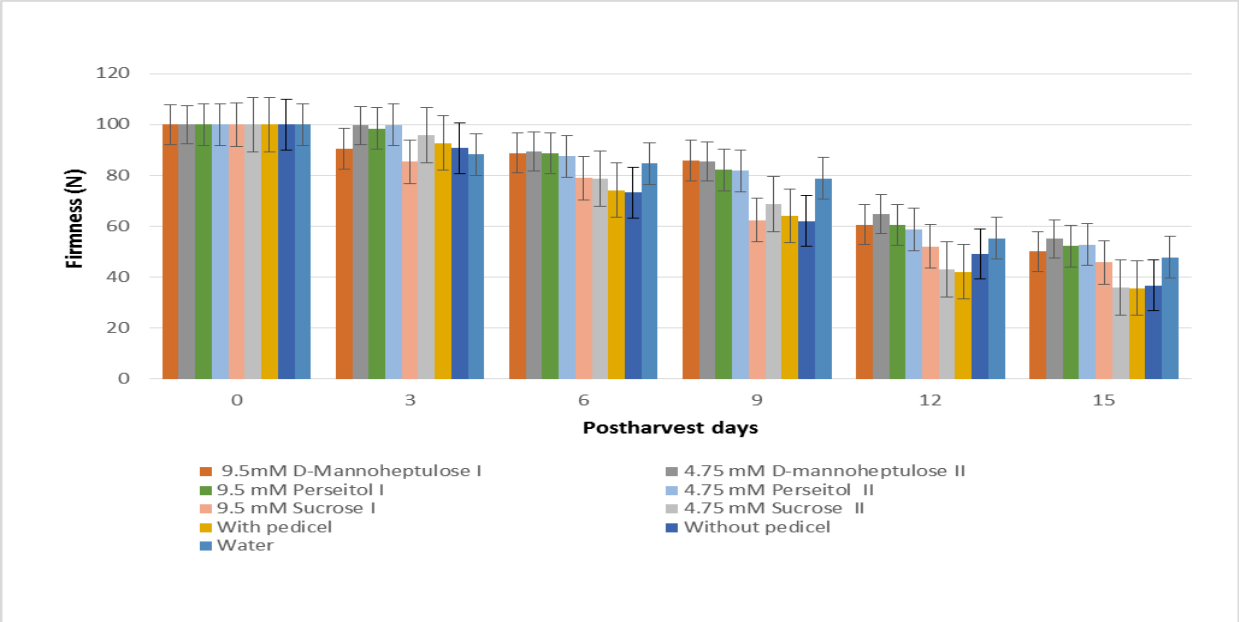

Figure 2: The interaction effect of C7 sugars postharvest infusion, on firmness of Early-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM (n=48) LSD $_{(P\ (0.05)}$ = 5.907

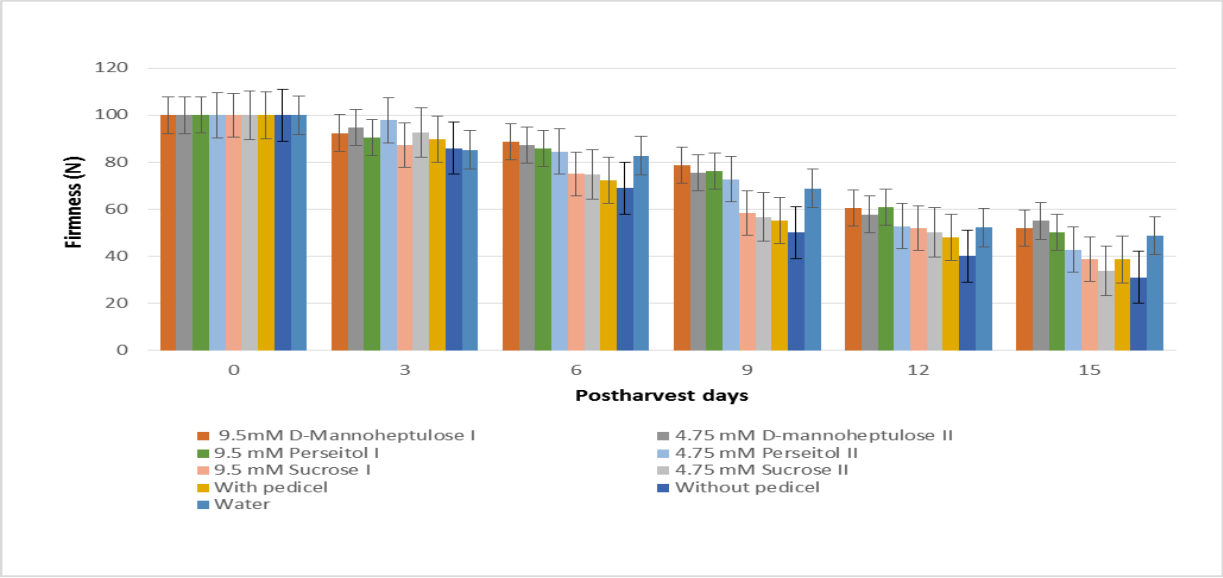

Figure 3: The interaction effect of C7 sugars postharvest infusion, on firmness of Early-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM (n=48). LSD $_{(P\ (0.05)}$ = 6.447

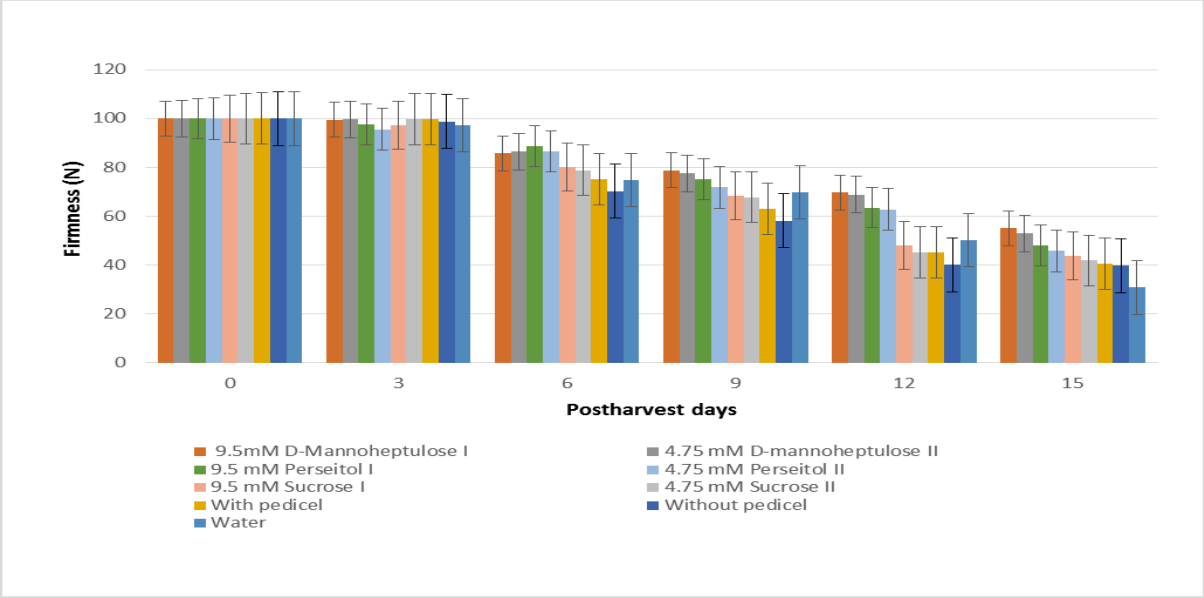

Figure 4: The interaction effect of C7 sugars postharvest infusion, on firmness of Mid-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM (n=48). LSD $_{(P\,(0.05)}$ = 8.671

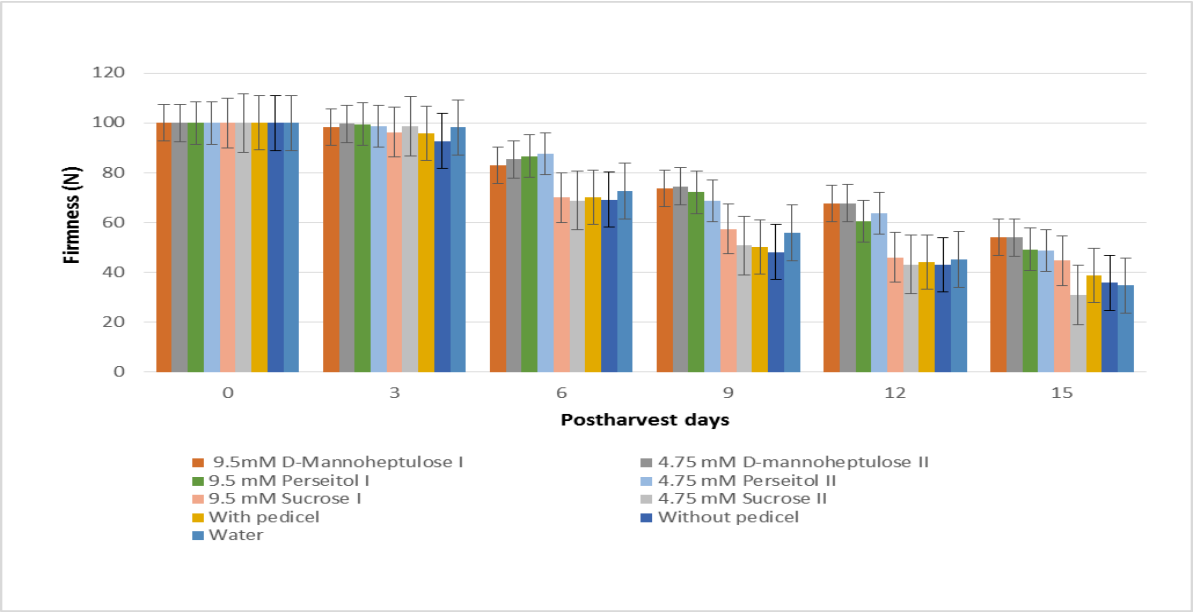

Figure 5: The interaction effect of C7 sugars postharvest infusion, on firmness of Mid-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM (n=48). LSD $_{(P\,(0.05)}$ = 7.883

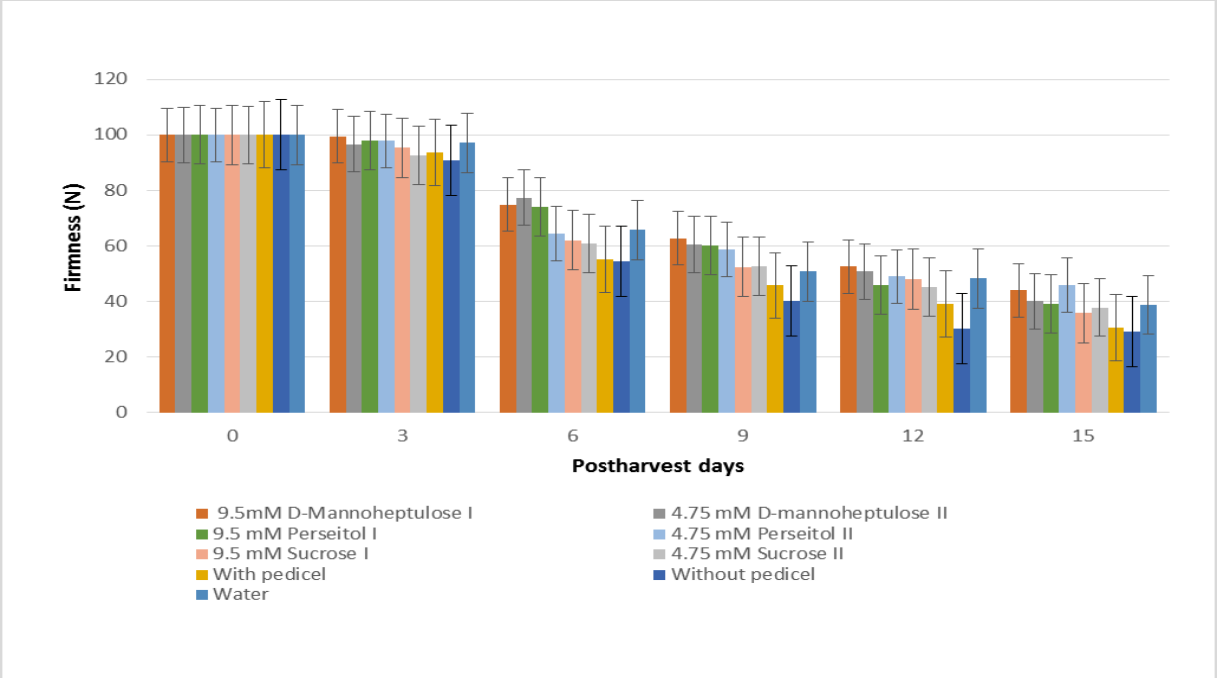

Figure 6: The interaction effect of C7 sugars postharvest infusion, on firmness of Late-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM (n=48). LSD $_{(P\,(0.05)}$ = 9.741

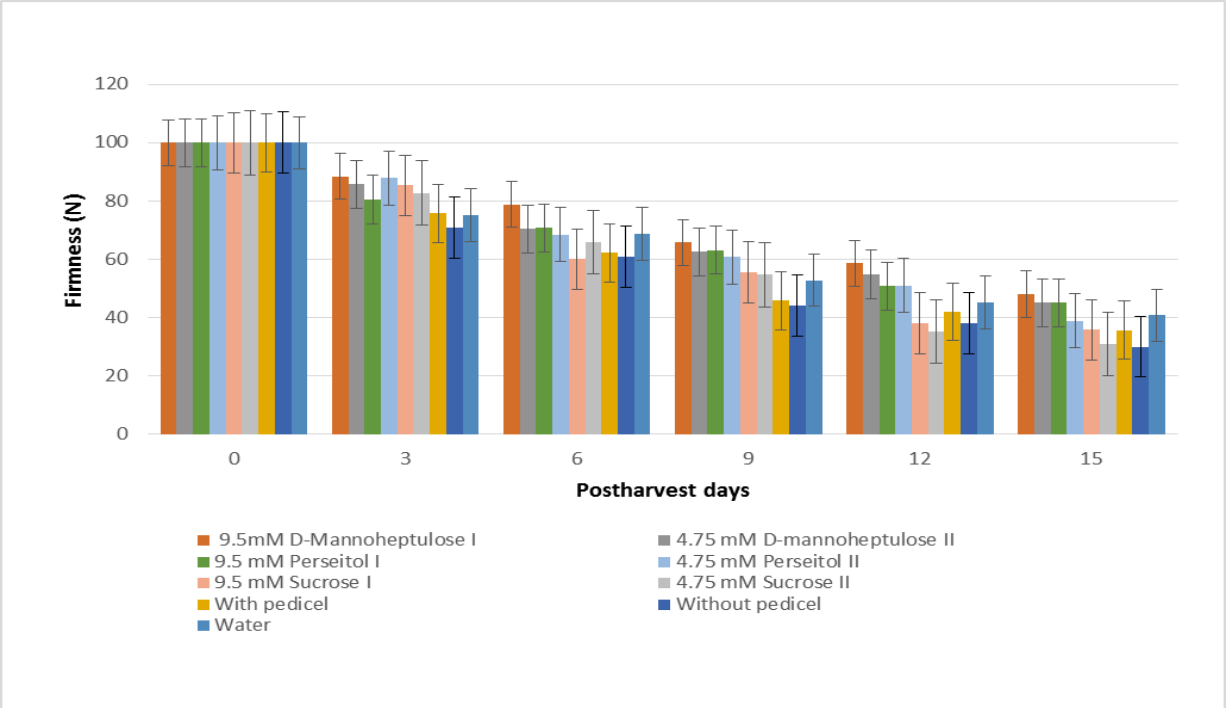

Figure 7: The interaction effect of C7 sugars postharvest infusion, on firmness of Late-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM (n=48). LSD $_{(P\,(0.05)}$ = 10.489

Respiration

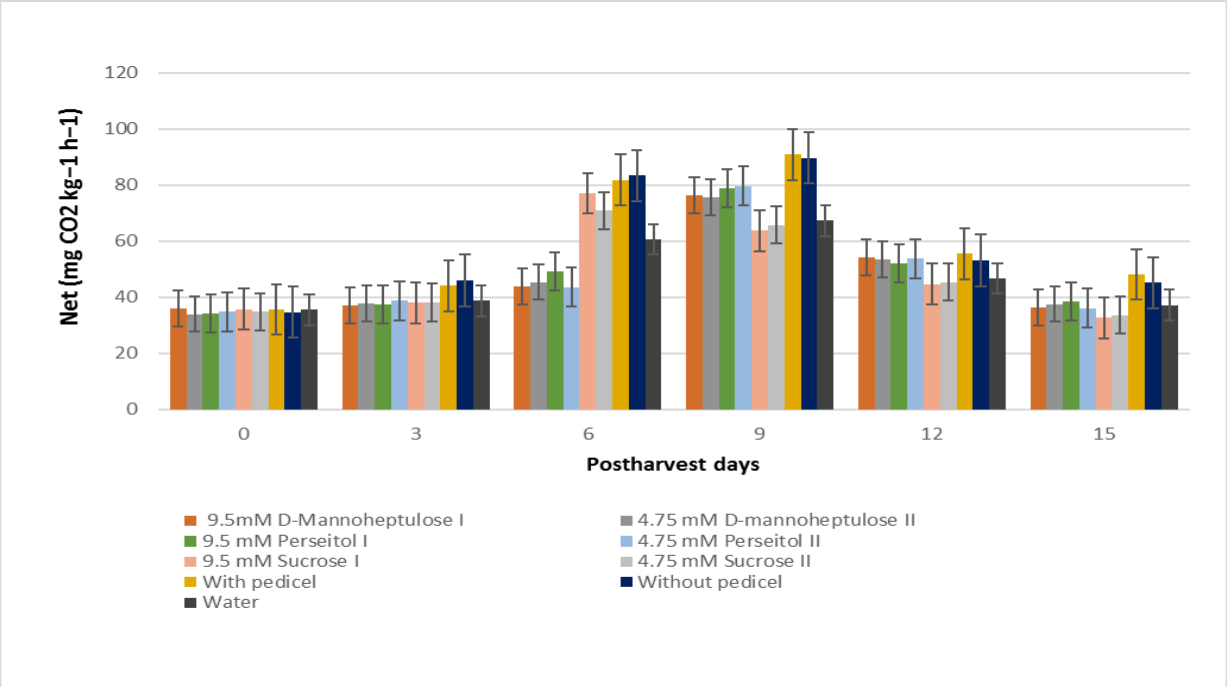

Figure 8: The interaction effect of C7 sugars postharvest infusion, on Net CO_2 production of Early-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM (n=48). LSD $_{(P\ (0.05)}$ = 9.796

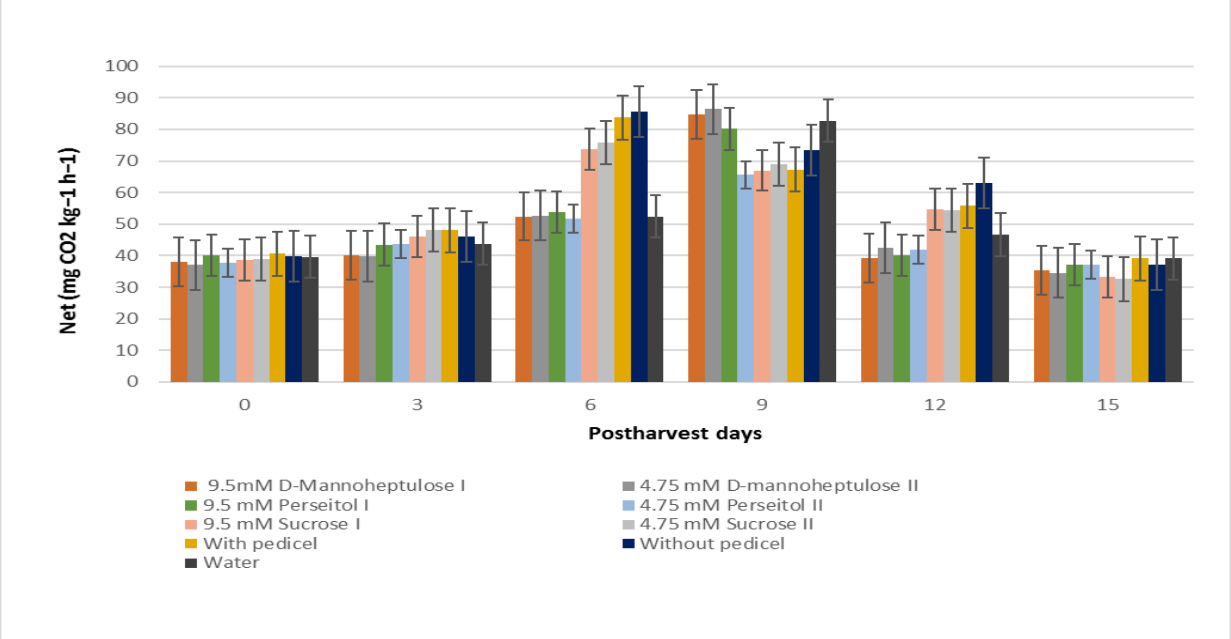

Figure 9: Interaction effect of C7 sugars postharvest infusion and net CO_2 production of Early-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM (n=48). LSD $_{(P\ (0.05)}$ = 10.849

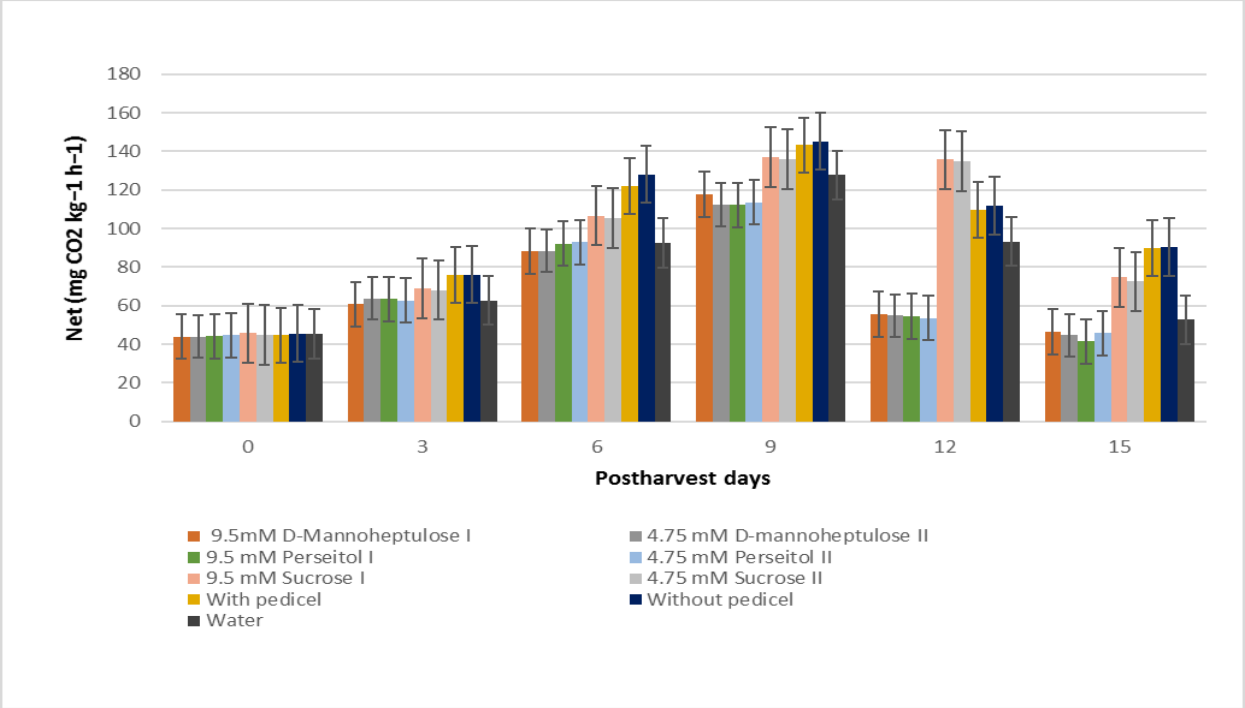

Figure 10: Interaction effect of C7 sugars postharvest infusion, on net CO_2 production of Mid-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM (n=48). LSD $_{(P\,(0.05)}$ = 7.445

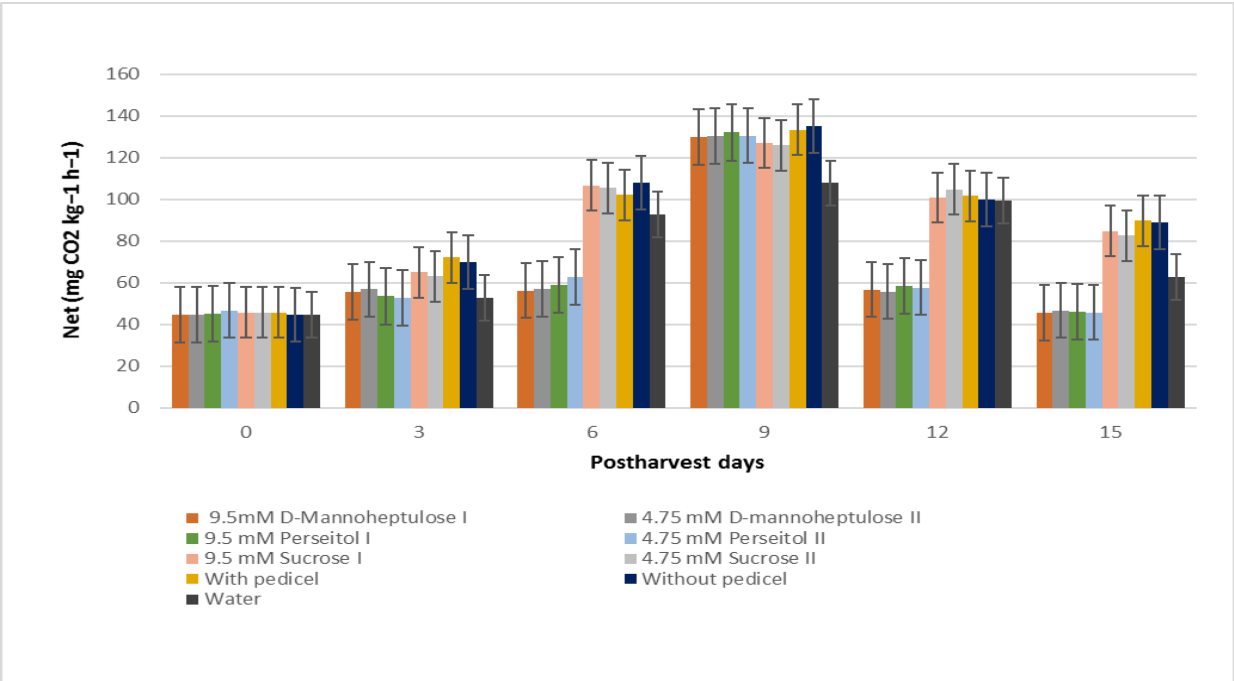

Figure 11: The interaction effect of C7 sugars postharvest infusion, on Net CO_2 production of Mid-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM (n=48). LSD $_{(P\,(0.05)}$ = 8.211

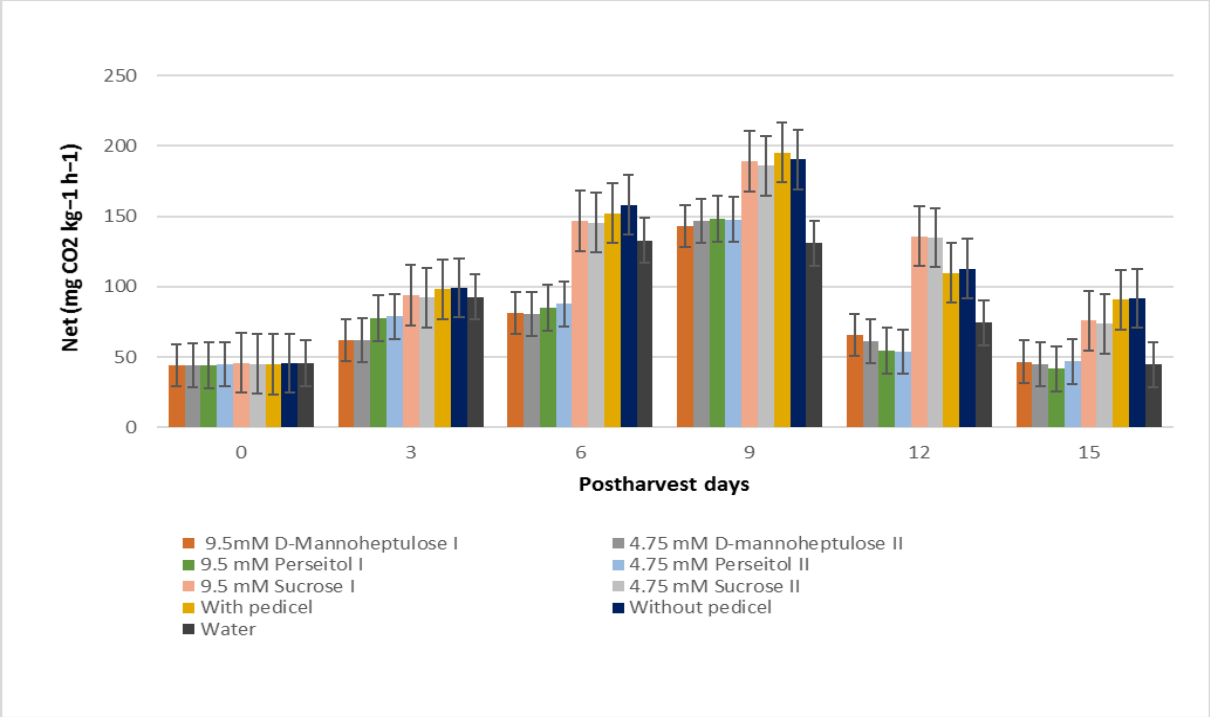

Figure 12: Interaction effect of C7 sugars postharvest infusion, on net CO_2 production of Late-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM (n=48). LSD $_{(P\ (0.05)}$ = 12.341

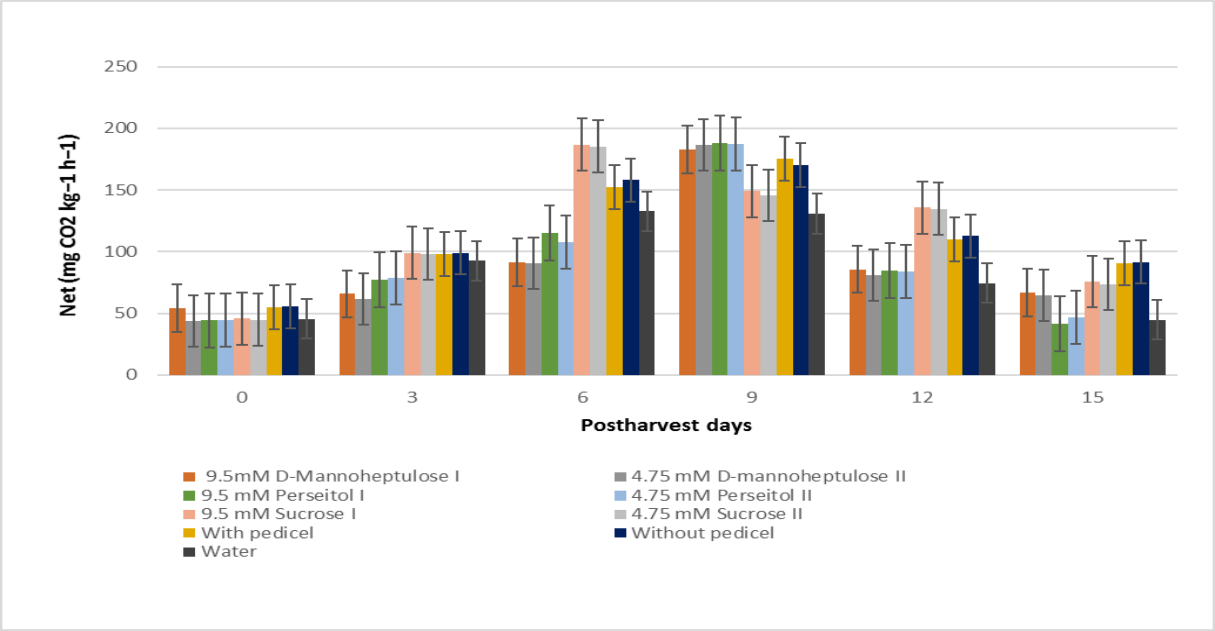

Figure 13: Interaction effect of C7 sugars postharvest infusion, on net CO_2 production of Late-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM (n=48). LSD $_{(P\ (0.05)}$ = 11.654

Internal Quality Assessment

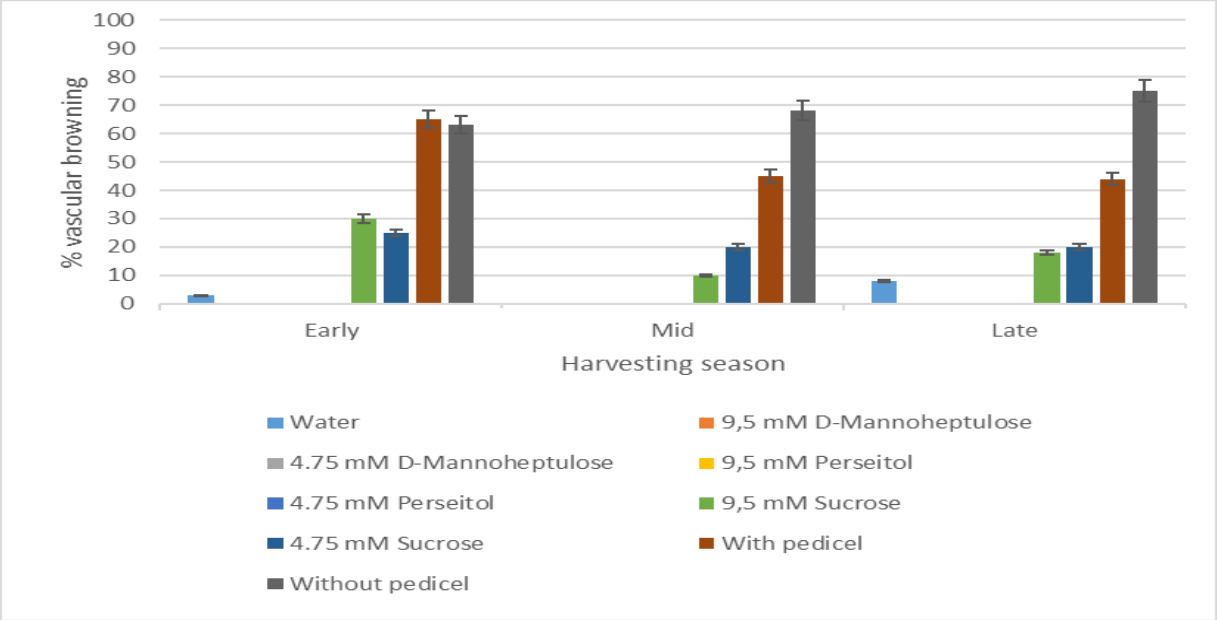

Figure 14: Effect of C7 sugars infusion and their interaction effect, on vascular browning of 'Hass' fruit from different harvesting seasons (early-, mid-, and late- season). Vertical bars represent ± SEM (n=48). LSD $_{(P\,(0.05)}$ = 3.662

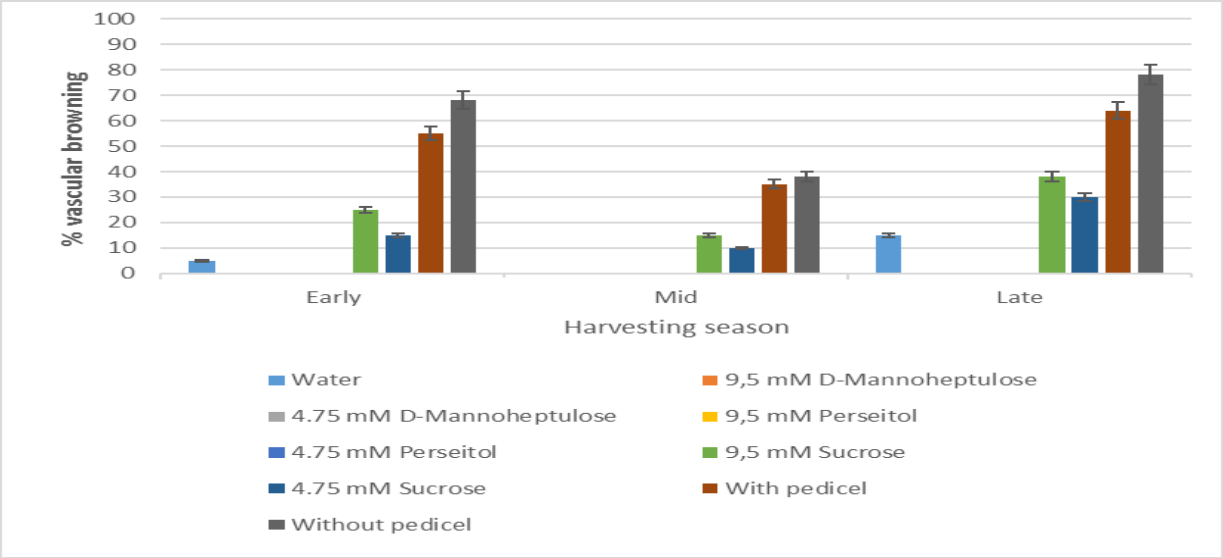

Figure 15: Effect of C7 sugars infusion and their interaction effect, on vascular browning of 'Fuerte' fruit from different harvesting seasons (early-, mid-, and late-season). Vertical bars represent ± SEM (n=48). LSD $_{(P\,(0.05)}$ = 2.841

External Quality Assessment

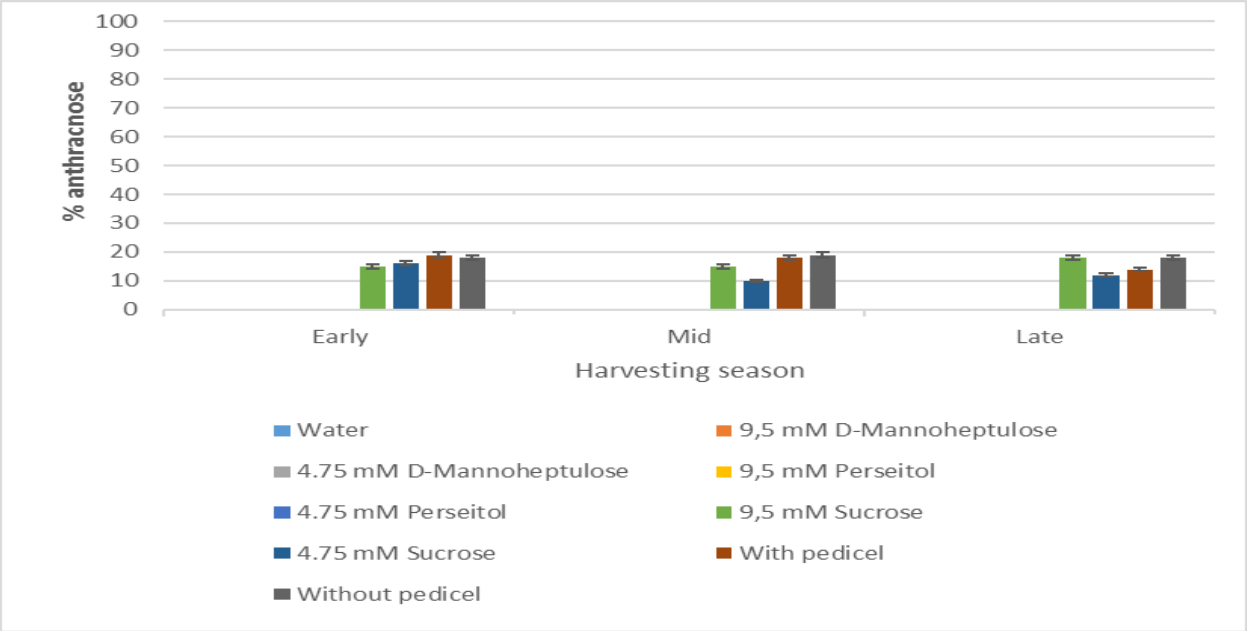

Figure 16: Effect of C7 sugars infusion and their interaction effect, on anthracnose infestation in different harvesting seasons (early-, mid-, and late-season) of 'Hass' fruit. Vertical bars represent ± SEM (n=48). LSD $_{(P\ (0.05)}$ = 2.111

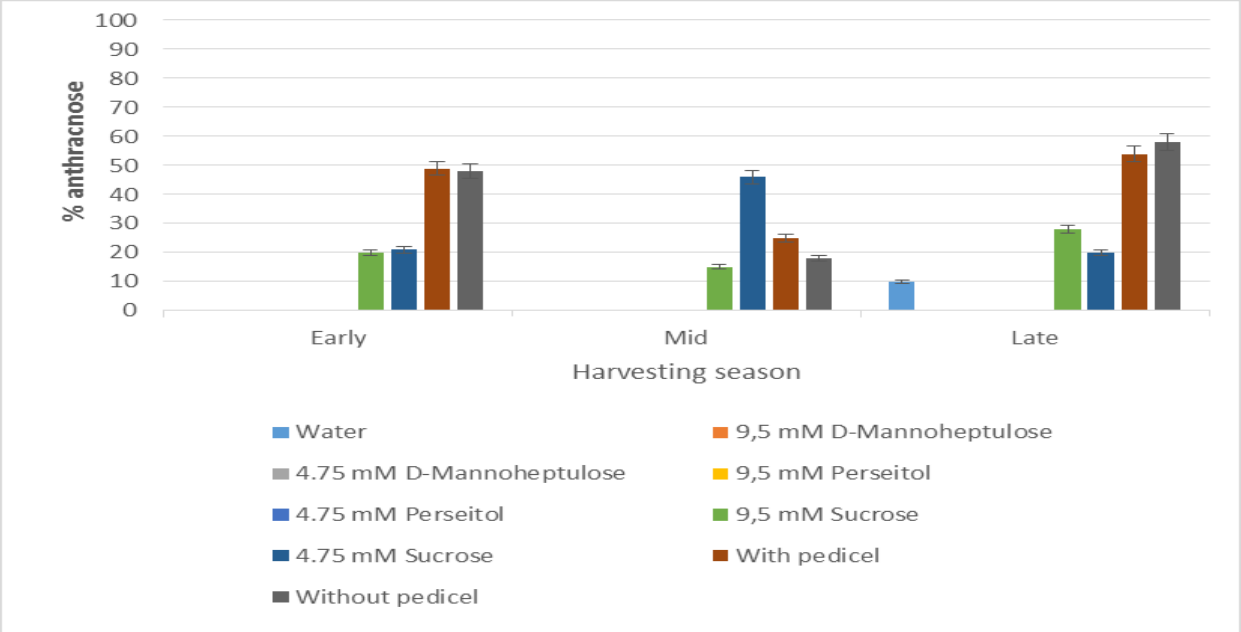

Figure 17: Effect of C7 sugars infusion and their interaction effect, on anthracnose infestation in different harvesting seasons (early-, mid-, and late-season) of 'Fuerte' fruit. Vertical bars represent ± SEM (n=48). LSD $_{P\ (0.05)}$ = 1.872

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CHAPTER 3

THE EFFECT OF C7 SUGAR INFUSION ON AVOCADO (*Persea americana* Mill.) FRUIT RIPENING AND ON FRUIT SUGAR AND OIL CONCENTRATION AT DIFFERENT MATURITY STAGES

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ABSTRACT

Avocado (*Persea americana* Mill.) is an important tropical fruit that is sought after due to its distinct attributes; it is highly nutritious, containing high amounts of thiamin, Vitamin E and monounsaturated fatty acids. Over the past decade it has been discovered that two heptoses, *D*-mannoheptulose and perseitol, that are found in vast amounts in the mesocarp tissue and that act as antioxidants. Although the functions of these sugars in plants remain unclear, there are many indications that these compounds contribute to fruit quality. Therefore, increasing the amount of the C7 sugar pool in avocado mesocarp was explored through simulation of a continuous sugar flow by attaching a silicon tube to the pedicel and infusing these sugars. The effect of C7 sugar infusion on fruit sugar concentrations, and the relationships between individual sugars, and oil content during fruit ripening were determined. 'Fuerte' and 'Hass' fruit were harvested from Bounty Farm, Winterskloof, KwaZulu-Natal Midlands, South Africa at three- picking seasons (early-, mid-, and late-harvest). Mesocarp tissue analysis was carried out after infusion of C7 sugars (*D*- mannoheptulose and perseitol) or the C6 sugar (sucrose). Infusion of C7 sugars (*D*- mannoheptulose or perseitol) positively influenced mesocarp mannoheptulose and perseitol levels at both infusions, while water-infusion and C6 sugar (sucrose) infusion had no influence on maintaining the levels of these C7 sugars. Similar trends of low mannoheptulose and perseitol concentrations were observed when fruit were infused with the C6 sugar (sucrose) and those that were not infused, whereby non-infused fruit during the late-harvest, showed a low level of C7 sugars concentration. For fruit harvested during the early-season, *D*-mannoheptulose and perseitol concentrations declined rapidly six days after postharvest infusion in C6 sugar (sucrose) infused fruit and control fruit (fruit with pedicel and fruit without pedicel) particularly in Hass. It was also noted that dry matter and oil content increased with fruit maturity, while moisture content decreased, resulting in faster ripening.

Firstly, C7 sugar infusion was a means to improve the mesocarp C7 sugar concentration; while, secondly, to investigate the effect of C7 sugar infusion on the concentration of fruit sugars at three different harvesting season. The C7 sugars seem to play a vital role in maintaining fruit quality and serve as an important source of energy in avocado fruit. The rapid decrease of these sugars may be an indication that maintaining a high level of these sugars is necessary to minimize postharvest losses through postharvest physiological disorders.

Keywords: *C7 sugar, C6 sugar, postharvest infusion, 'Hass', 'Fuerte', D-mannoheptulose, perseitol, harvesting-season, fruit quality, concentration, maturity, oil content, early-harvest, mid-harvest, late-harvest, moisture content, dry matter, mesocarp tissue.*

3.1 INTRODUCTION

Avocado is an oil-accumulating fruit that is highly susceptible to qualitative and quantitative postharvest losses (Bill *et al.,* 2014). The decision to harvest fruit, is based mainly on the oil content (Young and Lee, 1978). Tesfay *et al.* (2010) reported a decline in C7 sugars in the mesocarp during postharvest storage, in line with the previous suggestion that these uncommon sugars are associated with fruit quality (Bertling and Bower, 2005). Avocado mesocarp has large quantities of C7 sugars, particularly *D*-mannoheptulose, and its reduced polyol form perseitol (Liu *et al.,* 1999), as well as volemitol (Cowan and Bornman, 2004). Oil content is a key part of the sensory quality of avocado (Dreher and Davenport, 2013). During avocado fruit maturation, oil and dry matter accumulate (Ozdemir and Topuz, 2004). As the oil accumulates in the mesocarp, its water content declines, by the same amount so that the total percentage of oil and water content remains constant during fruit life (Gaydou *et al.*, 1987). The biosynthesis of lipids in oily fruit has been reviewed, and acetyl-CoA has been confirmed to be the precursor for *de novo* fatty acid biosynthesis (Salas *et al.,* 2000). Several treatments have been employed as means to achieve successful fruit quality maintenance postharvest. Blakey *et al.* (2009) reported the infusion of aqueous ABA solution and water into avocado resulting in faster flesh softening, reducing the spread of days to ripening. Avocado growers are faced with severe logistic problems due to the inconsistent fruit quality aligned with the ripening physiology of avocado fruit (Rose, 2003). By supplementing the 'sugar pool' of the fleshy mesocarp with C7 sugars, which act as antioxidants (Tesfay *et al.,* 2010) and, possibly, as respiratory substrates (Tesfay *et al.,* 2012), ripening might be inhibited. The reduction of these uncommon C7 sugars, particularly *D*-mannoheptulose and perseitol below a threshold of $\approx$20 $mg.g^{-1}$ DM could be a physiological prerequisite for fruit ripening (Liu *et al.,* 2002). This may indicate that the ripening process is associated with catabolism of C7 sugars, but it is equally possible that the C7 sugars themselves may be controlling the ripening process. The potential involvement of

these C7 sugars in inhibiting ripening when the fruit is detached from the tree is, however, still not clear. This study attempts to investigate, if the simulation of the fruit being attached to the tree (by providing a constant flow of CHOs through infusion of water, sucrose or the C7 sugar *D*-mannoheptulose or perseitol) can inhibit ripening. The hypothesis is that maintaining a physiological *D*-mannoheptulose or perseitol concentration and mesocarp water content in the fruit mesocarp delays fruit ripening.

3.1.1 Research aim

The main aim of this experiment was to investigate the effect of postharvest C7 sugars infusion on the changes in mesocarp sugar concentration and the relation between individual sugars and oil content during ripening for the three maturity stages as defined by picking seasons (early-, mid-, and late-season fruit).

3.1.2 Research objectives

3.1.2.1 To investigate, if infusion of the C7 sugars *D*-mannoheptulose and perseitol through the pedicel can maintain physiological concentrations of the C7 sugars *D*-mannoheptulose and perseitol within the fruit.

3.1.2.2 To investigate the effect of mesocarp moisture content in relation to C7 sugars concentration during postharvest ripening.

3.1.2.3 To investigate, if the continued supply of *D*-mannoheptulose, perseitol and water can delay mesocarp deterioration and maintain fleshy fruit mesocarp.

3.1.2.4 To investigate the effect of C7 sugars infusion on the oil content of individual fruit harvested at three different maturity stages as defined by picking season (early-, mid- and late-season fruit).

3.2 MATERIALS AND METHODS

3.2.1 Fruit material and experimental design

The research was carried out in the 2015 and 2016 avocado growing season, using physiologically mature 'Hass' and 'Fuerte' fruit. During these seasons fruit were collected from mature 'Hass' and 'Fuerte' avocado orchards of Bounty Farm, Winterskloof, in the KwaZulu-Natal Midlands, South Africa (29°28´S; 30°161´E). Fruit were harvested with about 5cm pedicels retained and selected based on uniformity of shape, colour and size. Only bruise- and blemish-free fruit were used in the experiment. To prevent physical damage, harvested fruit were gently placed into crates and immediately transported, to the Horticultural Science laboratories at the University of KwaZulu-Natal, Pietermaritzburg. Three separate experiments were carried out during the 2015 and 2016 season, in an early, mid- and late- harvesting season. Avocado fruit within a mass of 203-243 g (class 1; Government Gazette 37223, 17 Jan 2014) were harvested on 29/04/2016 ('early', moisture content 72%, equivalent to 28% DM and 25% oil), on 09/09/2015 ('mid', moisture content 66%, equivalent to 34% DM and 26 % oil) and on 01/12/2015 ('late', moisture 60% moisture, equivalent to 40% DM and 31 % oil), representing the early-, mid- and late-harvesting season, respectively. For each experiment a total number of 96 avocados with 5 cm pedicel were acquired, consisting of 48 'Hass' and 48 'Fuerte' fruit. Fruit were randomly divided into nine treatments, seven infusion treatments (C7 sugars solutions 1.5 mL of 9.5 mM/fruit; 4.75 mM/fruit *D*-Mannoheptulose, 9.5 mM/fruit; 4.75 mM/fruit Perseitol solution) was infused to 6 fruit per treatment. The C6 sugar solutions of sucrose with 9.5 mM/fruit; 4.75 mM/fruit were infused in 1.5 mL pedicel. Water of 1.5 mL was infused in six fruit and two untreated fruit categories consist of six fruit each, one with 2cm pedicel and one without pedicel).

3.2.2 Chemicals

All chemicals were obtained from Sigma-Aldrich®, Saarchem®, Fluka® or Glycoteam GmbH.

3.2.3 Treatments

Silicon tubing was attached to the pedicel (re-cut to about 2cm prior to treatment to remove dead tissue) of fruit, and sealed with petroleum jelly at the base (Cutting and Bower, 1987). According to Bertling *et al.* (2011). An amount of 1.5 mL water or sucrose (C 6 sugar) or 9.5 mM or 4.75 *D*-mannoheptulose or perseitol was infused into six fruit per treatment. Control fruit (with and without pedicel) remained untreated.

3.2.4 Data collection and sample preparation

Avocado fruit are highly heterogeneous, so in order to minimize sampling variation, two core samples (2.5 ml each) were taken along the equatorial region of the fruit using a 15 mm diameter cork-borer (Meir *et al.,* 1991); sampled areas on each fruit were immediately sealed with petroleum jelly to prevent mesocarp oxidation. For each experiment as defined by harvesting season fruit were assessed on sugars content, moisture content and oil content, throughout shelf-life. Samples of both cultivars, 'Hass' and 'Fuerte', were sampled and measured in three (3) day intervals of (day 0, 3, 6, 9, 12, and 15) during the C7 postharvest infusion; two core tissue samples were immediately lyophilized and stored at -20 ℃ until further analysis.

3.2.5 Determination of soluble sugar concentration

Freeze-dried, ground material (0.05 to 0.10 g DM) was mixed with 10 mL 80 % (v/v) ethanol and homogenized for 60 s. Thereafter, the mixture was incubated in an 80 ºC water bath for 60 min and kept at 4 ºC overnight according to Tesfay *et al.* (2010). After centrifugation at 12,000 x *g* for 15 min at 4 ºC, the supernatant was filtered through glass wool and was taken to dryness in a Savant Vacuum Concentrator (SpeedVac, Savant, New York, USA). Dried samples were re-suspended in 2 mL ultra-pure water, filtered through 0.45 μm nylon filters and analysed using High-performance liquid chromatography (HPLC) (LC – 20AT, Shimadzu Corporation, Kyoto, Japan) system to which a refractive index detector (RID-10A, Shimadzu Corporation, Kyoto, Japan) was attached. The elution was isocratic, using ultrapure water as the mobile phase. Individual sugars were identified by co-elution with standards of sucrose (Sigma-Aldrich, St Louis, Missouri, USA), mannoheptulose and perseitol (Glycoteam, Hamburg, Germany). Sugars were quantified by using a standard curves for each sugar. Samples were analysed twice and the mean taken.

3.2.6 Percentage mesocarp moisture content (MC) and dry matter (DM)

The MC was determined by measuring the difference in mass of the sample taken from the equatorial region of the fruit before and after lyophilisation. Thereafter, the % moisture content was determined as follows:

Moisture content (%) = $(M_0\text{-}M_1/M_0) \times 100$

Where:

M_1 = Final mass of the dried sample

M_0 = Initial mass of the fresh sample

Dry matter (DM) was determined by freeze-drying a sample at 60 ºC to a constant mass. The final and initial mass difference was used to calculate dry matter percentage, taking a representative sample of mesocarp of avocado tissue as described by Meyer and Terry (2008).

3.2.7 Measurement of mesocarp oil content

Mesocarp oil content was measured from ground, lyophilised sample material and quantified using the method described by García *et al.* (2009), with slight modifications. Hexane (9.0 mL) was added to 300 mg lyophilised mesocarp tissue in a test tube which was placed into an ultra-sonic bath for 10 min. The sample was filtered under vacuum and another 6 mL hexane added to the test tube. This solution was left to stand for 5 min and the tube emptied into the Buchner funnel. The test tube was then rinsed with 3 mL hexane. The 18 mL of hexane was combined and dried using a GeneVac® concentrator (SP Scientific, Genevac Limited, IPSWICH ENG.). The recovered oil was weighed and the percentage oil content was calculated using the following equation and expressed as [% (w/w)].

$$\text{Oil content (\% w/w)} = \frac{\text{dry matter (\%) x oil mass (g)}}{\text{dry pulp mass (g)}} \quad (1)$$

3.2.8 Statistical analysis

Data were analyzed in the form of a factorial design, where each treatment combination consisted of six fruit, each fruit constituting a single replication. Analyses of variance were performed using GENSTAT (edition 17th; VSN International, Hemel Hempstead, UK). Standard deviations (S.D.) were calculated and differences among treatments were separated by a significant difference (LSD) test, with $P \leq 0.05$ regarded as significant. The following parameters were used to evaluate the change in the quality of the avocados during ripening: soluble sugar concentration, mesocarp moisture content (MC), % dry matter (DM), and oil concentration.

3.3 RESULTS

3.3.1 Sugar Analysis- Mannoheptulose and Perseitol

There were significant differences ($P < 0.005$) in infusion treatment means with regard to the concentrations of *D*-mannoheptulose and perseitol (mg g^{-1}) in the mesocarp tissue of 'Fuerte' and 'Hass' avocado fruit harvested in three different season (*i.e*., early-, mid-, and late-harvest). Fruit infused with C7 sugars (*D*-mannoheptulose and perseitol) had the highest concentration of these sugars; mannoheptulose was the predominant sugar in fruit from the early harvest, followed by mid-harvest; endogenous mesocarp concentrations, however, declined throughout the progress of postharvest infusion (Fig 1-4). Infusion of C7 sugars was able to maintain the initial levels of these sugars (*D*-mannoheptulose and perseitol). Water infusion had an effect in maintaining C7 sugars during postharvest infusion, by maintaining the mesocarp mannoheptulose concentration levels, but the difference to sucrose infusion was not significant during day nine, since there was, 7.642 mg g^{-1} as difference between mean values of water infused fruit and 7.729 mg g^{-1} as difference between mean values of sucrose- infused fruit, with LSD ($p \leq 0.05$) = 13.642 (Fig 6). Control fruit had the lowest mesocarp *D*-mannoheptulose (Fig. 1-6) and perseitol (Fig. 7-12) concentrations. The effect of harvest-season on C7 sugars was found to be significant (LSD ($p \leq 0.05$) = 1.621), the mannoheptulose trends (Fig. 1-4) illustrate a high amount of mannoheptulose in early-harvest and mid- harvest fruit, which seems to indicate that C7 sugars play a major role during this part of fruit development. During the late season, however, the perseitol concentration was higher than that of *D*-mannoheptulose, suggesting that this sugar alcohol is related to fruit storability; this is also supported by the fact that the 'Hass' mesocarp had significantly higher concentration of perseitol than the mesocarp tissue of 'Fuerte' (Fig. 11-12) and the postharvest quality of 'Hass' is known to outperform that of 'Fuerte' (Bill *et al.,* 2014). Following six days of postharvest infusion, *D*-mannoheptulose and perseitol concentrations seemed to drastically decrease in mesocarp

tissue. The *D*-mannoheptulose concentration on storage day nine was similar to, and in some cases lower than, that of perseitol, suggesting that, during postharvest storage, *D*-mannoheptulose is utilised more readily as a carbohydrate than perseitol. Even at day 12, C7 sugars (*D*-mannoheptulose and perseitol) dominated over the C6 sugars (data not presented), indicating a lesser importance of this sugar in avocado. It was also noted that the concentrations of glucose increased after 6 days of postharvest days of infusion, while sucrose declined slightly during ripening. *D*-mannoheptulose and perseitol, as well as glucose and sucrose, were consistently present during ripening in all harvesting seasons.

3.3.2 Moisture Content (MC) and Dry Matter (DM)

Infusion treatment and harvesting season significantly affected avocado MC and its reciprocal value, DM. Dry matter increased with a decrease in moisture content, while the postharvest infusion was performed on fruit of three different seasonal pickings (*i.e.*, early-, mid-, and late-harvest, Fig. 13-18). A comparison of the different postharvest infusion treatment means indicated that the decrease in the MC occurred at a slower rate during day 0, 3, and 6 of early harvested fruit. In terms of mesocarp MC, trends tend to decrease with postharvest days (Fig. 13-18). A general reduction in the MC was observed in all avocado fruit for both, 'Hass' and 'Fuerte' fruit. Mesocarp MC dropped from 77.0 to 66.0% from early- to mid-harvest, and 71.6 to 64.0% from mid- to late-harvest in 'Fuerte', whereas in 'Hass' it dropped from 78.0 to 67.0% from early- to mid-harvest, and 75.6 to 65.2% from mid- to late-harvest season. Likewise, mesocarp dry matter content also increased with every harvest from early to late-season. Dry matter increased with increasing fruit maturity in both cultivars, 'Hass' and 'Fuerte' (Fig. 13-18).

3.3.3 Oil content

Harvesting season had a significant effect on the concentration of oil in fruit ($P<0.005$), and the interaction between infusion treatment and days postharvest was significant. The oil concentration increased slowly from early- to mid-season, but faster mid- to late season (Fig. 25-29). Early-harvested fruit had the lowest oil concentration, particularly in 'Hass' fruit (Fig. 3.25). These is probably due to the higher percentage moisture content at that sampling time. Overall, in the three harvesting season (*i.e*., early- mid- and late-harvest), *D*-mannoheptulose and perseitol infusions resulted in the highest mesocarp oil concentrations; however, after 12 days of postharvest infusion, there significant difference between C7 sugars and C6 sugar. Sucrose-infused fruit tended to contain a high amount of oil during early days of infusion.

3.4 DISCUSSION

3.4.1 C7 sugars in plants with special reference to mannoheptulose and perseitol

C7 sugars have various roles in plant (Rolland *et al*., 2002; Liu *et al.,* 1999), with rather diverse functions in avocado (Richings *et al*., 2000; Tesfay, 2009). The individual sugar concentration in the avocado fruit can be influenced by the type of cultivar and the type of fruit tissue (Landahl *et al*., 2009). As glucose and fructose concentrations remain at a low level throughout ripening (data not presented), it is suggested that the two predominant heptoses are utilised during the ripening process. Landahl *et al*. (2009) reported a significant difference in *D*-mannoheptulose and perseitol concentrations in different mesocarp sections. The reduction of C7 sugars, as maturity increases, led to the suggestion that these peculiar C7 structure can represent the main energy source in avocado fruit (Liu *et al*., 1999). The drastic reduction of the C7 sugar concentration appears after postharvest storage and ripening (Blakey *et al*., 2009). Similar to the present study Bertling and Bower (2006), reported 'Hass' mesocarp tissue to have a higher concentration of *D*-mannoheptulose than 'Fuerte', with concentrations in both

cultivars decreasing with fruit maturity. Furthermore, C7 sugar concentrations also differ with cultivar (Bertling and Bower, 2005) and both, maturity and a cultivar affect postharvest fruit quality (Bertling and Bower, 2006). Tesfay *et al.* (2010) reported a higher concentration of mannoheptulose in the mesocarp tissue of 'Hass' avocados than perseitol at the time of harvest, as was found in this study after three days of postharvest (Fig. 1-12). Perseitol has been postulated as the C7 sugar storage compound (Tesfay, 2009); the present results during late season concur with this, as there was high levels of perseitol during 3 days of postharvest infusion.

This may also suggest that there was high amount of perseitol at harvest. Only the infusion of C7 sugars had a significant influence on fruit mannoheptulose and perseitol sugar levels. The results indicate a significant loss in mannoheptulose during the late-harvest infusion and this may be due to conversion of mannoheptulose to perseitol (Tesfay, 2009) or this C7 sugar alcohol after conversion is stored as a storage compound in the avocado cotyledon (Tesfay *et al.,* 2011). However, this may also suggest that during the late harvest stage there is a disturbance of sugar solutes transportation from the seed through the seed coat into the fleshy mesocarp, due to senescence of the seed coat (Cowan *et al.,* 1997), and this concurs with the present results as it was observed a significant decline of *D*-mannoheptulose after 9 days of postharvest infusion during late-harvest (Fig. 5-6). Confirming Liu *et al.*'s (1999) findings that C7 sugars are forming a more important group of carbohydrates in avocado than C6 sugars. Sucrose declined slightly during ripening (data not presented), which is suggested to relate with an increase in cellulase activity breaking down cell walls and forming glucose. As expected, due to a continuous supply of C7 sugars during postharvest through pedicel infusion, resulted in a subsequent reduction of metabolic rate activity that results in suppression of ripening. A phenomenon previously reported by Bertling and Tesfay, (2011) in 'Hass' avocados. These

data confirm that mannoheptulose and perseitol are the dominant sugars present during ripening.

3.4.2 Moisture Content and Dry Matter analyses

The inverse relationship that exists between the moisture content and the mesocarp oil concentration of avocado (Slater *et al.,* 1975), was confirmed in the present study (Figure 13-24). This decline in mesocarp moisture content have been previously observed (Osuna-Garcia *et al*., 2010; Yousef and Hasseine, 2010). During fruit maturation, there is an accumulation of dry matter and oil concentration in the fruit, while moisture content decreases, resulting in the overall increase in palatability (Osuna-Garcia *et al*., 2010). Water content has been considered as the easiest factor to measure in avocado (Bower, and Cutting, 1988). In South Africa, mesocarp moisture (water content) is used as a tool for maturity indexing, and also for the determination of the commencement of the picking period from start, indicating the time from which onwards fruit ripen normally, becoming palatable without shrivelling. This recommended mesocarp moisture content lies in the range of 69 to 75%, depending on the cultivar (Mans *et al.,* 1995; Hofman *et al.,* 2002). Bower *et al.* (2007) also found that water was the single most important factor in the variation of ripening of avocado fruit. However, untreated fruit depicted a significant decrease in MC, particularly in early-harvested which agrees with Munzhedzi, (2016) findings that 'Fuerte' fruit had low moisture content in early-harvest and this may be due to low dry matter content and the inverse relationship between oil content and moisture content (Kruger *et al.,* 1995).

3.4.3 Oil content

The mesocarp oil content of freshly harvested fruit remained fairly constant during the early- and mid-season and only showed a significant increase during the late-season, possibly this indicate an increased cell wall degradation leading to oil accumulation (Mostert *et al.,* 2007; Meyer and Terry, 2008), since C7 sugars may be possible precursor for fatty acid synthesis. This may result in a reduction in antioxidant *D*-mannoheptulose. However, oil content differences in avocado fruit depends on several factors, such as the type of the cultivar (Dodd *et al.,* 2010; Orhevba and Jinadu, 2011) and fruit development stage (Ozdemir and Topuz, 2004; Villa-Rodriguez *et al.,* 2011), as demonstrated by the present findings, with late-harvested fruit had high oil content followed by middle-harvested fruit, whereas Hass had high oil concentration in middle-harvest compared to Fuerte (Figure 28 - 30), possible this indicate the importance of the C7 sugars for internal quality and palatability of the fruit. The postharvest infusion had significant effect on oil content. The results concurs with Lee *et al.,* 1983, reports that oil mesocarp concentration does not change with time after harvest, but this variation has been observed across harvest season. Sucrose-infused fruit tended to contain a high amount of oil during early postharvest days after infusion. This may suggest that it contributes significantly to oil synthesis as it consists of glucose that is indirectly involved in fatty acids synthesis through by-product of glycolysis. This is why, there was no lower oil content since 9.282 % as difference between mean values of water infused fruit and 6.973 % as difference between mean values of sucrose- infused fruit, with LSD ($p \leq 0.05$) = 4.374, (Fig 30).

3.5 CONLUSION

In conclusion, the continuation of the *D*-mannoheptulose, perseitol and water supply through the pedicel infusion influences ripening and maintains a high concentration of these sugars in the mesocarp. Study concurs with Blakey *et al.* (2009), who reported fruit water content can

influence ripening, and C7 sugars to be a major carbon source and antioxidant in avocado fruit (Tesfay *et al.*, 2010). Indeed, C7 sugars may be a critical factor in avocado ripening physiology, and an important energy source (Liu *et al.*, 1999). Fruit maturity serves as a major determinant of final fruit quality (Kader, 1999). The season of picking influences the C7 sugar pool in mesocarp tissue, it has been confirmed that there is reduction in C7 sugars as picking is delayed. From the present findings, MC and DM are related to C7 sugars accumulation; it is, therefore, suggested that an increase in C7 sugars and water loss during ripening trigger the decline in the size of the C7 sugars pool. Fruit use up the C7 sugars which are antioxidants (important to avoid browning), then they are also precursors, maybe for the oil synthesis, so are needed for proper 'creaminess' of the fleshy mesocarp. It might also be, that water loss directly impacts on the transporting ability of the sugars, meaning as soon as fruit reach a certain level of dry matter, the sugars stay in the relevant fruit tissues and cannot be moved to other fruit parts. The maintenance of these C7 sugars during postharvest storage, to limit the loss of these sugars, is of paramount importance to reduce the risk of fruit not achieving eating ripeness and developing internal disorders. It should also be examined, if and how the depletion of C7 sugar levels postharvest can be reduced, and if the levels of the sugars can be manipulated before harvest to commence fruit ripening postharvest with a high C7 sugar pool to hopefully achieve high fruit quality.

FIGURES

Sugar profile (*D*-mannoheptulose)

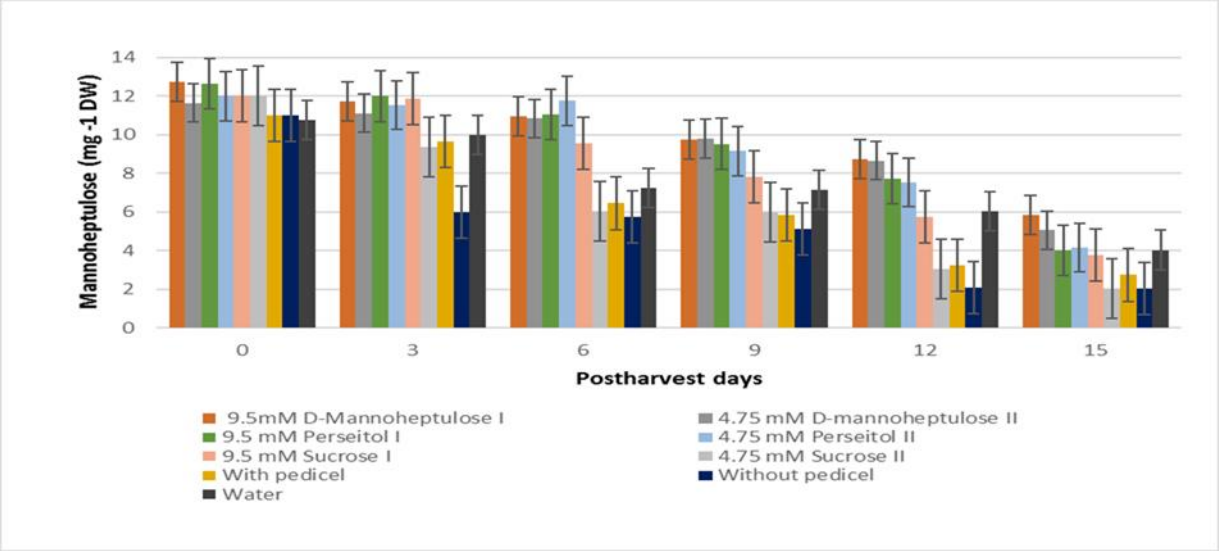

Figure 1: Effect of C7 sugars postharvest infusion treatment, on mesocarp *D*- mannoheptulose concentration of Early-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\,(0.05)}$ = 8.793

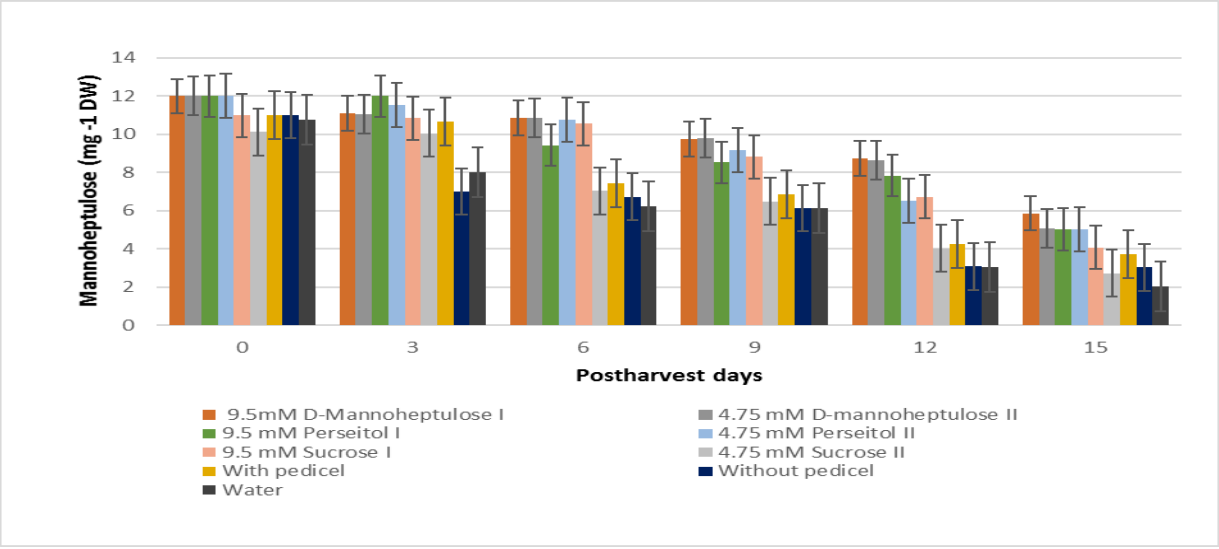

Figure 2: Effect of C7 sugars postharvest infusion treatment, on mesocarp *D*- mannoheptulose concentration of Early-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\,(0.05)}$ = 7.936

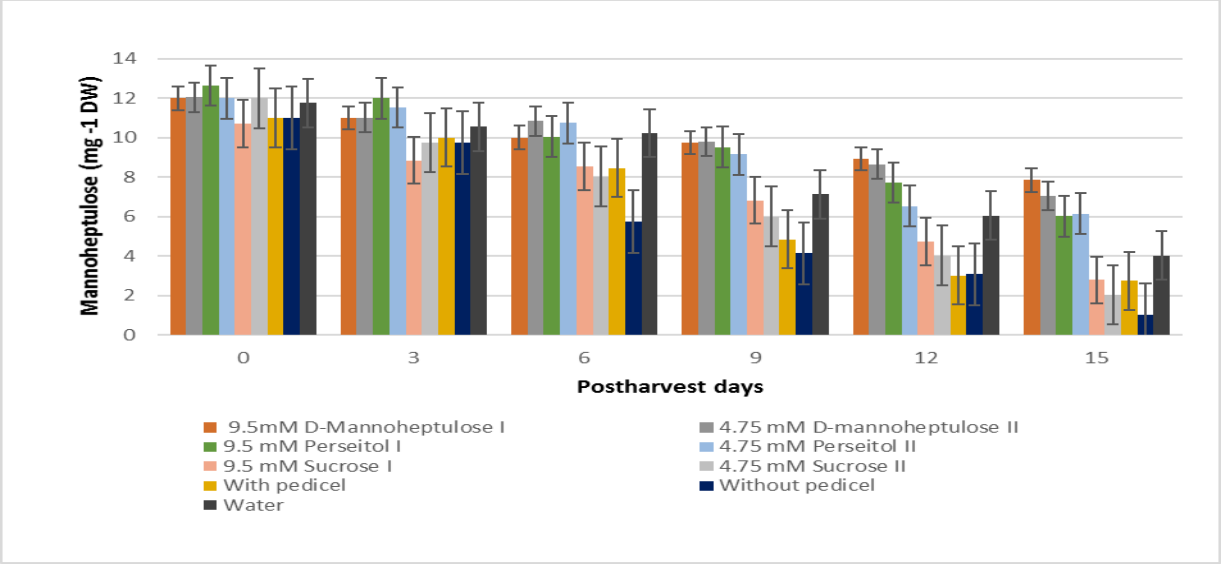

Figure 3: Effect of C7 sugars postharvest infusion treatment, on mesocarp *D*- mannoheptulose concentration of Mid-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\,(0.05)}$ = 10.11

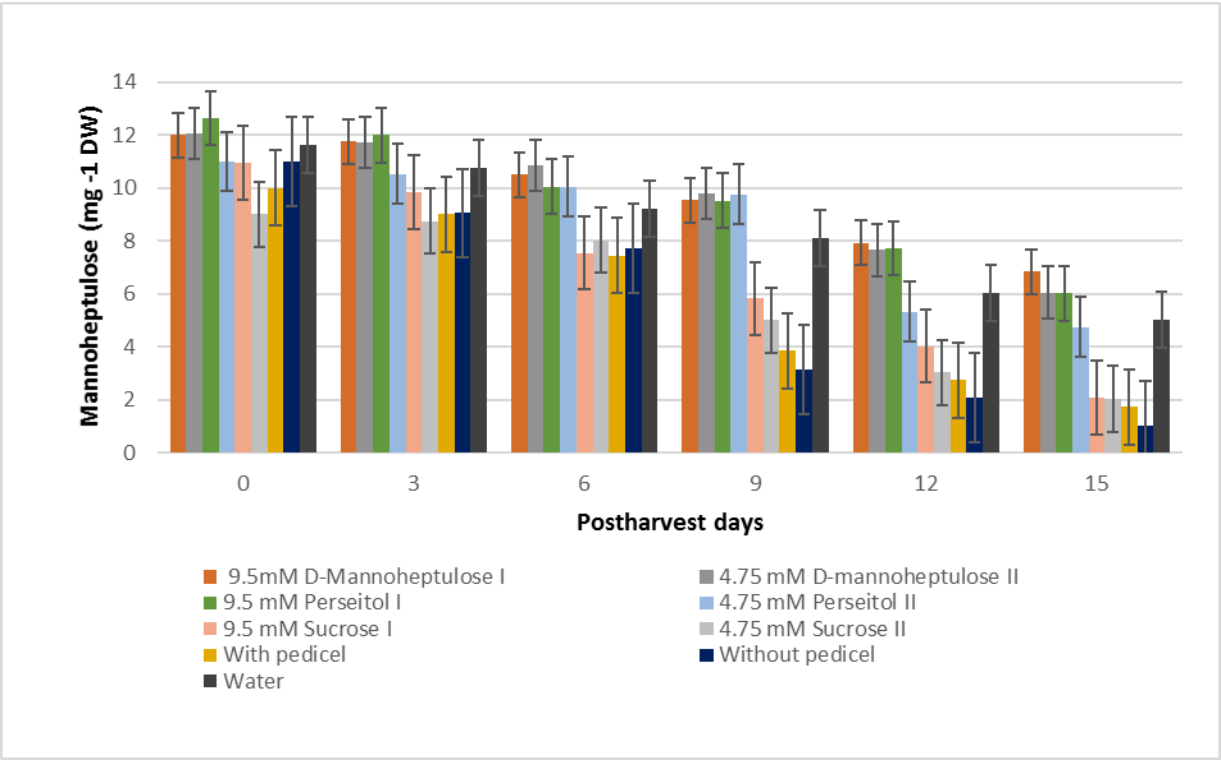

Figure 4: Effect of C7 sugars postharvest infusion treatment, on mesocarp *D*- mannoheptulose concentration of Mid-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\,(0.05)}$ = 11.002

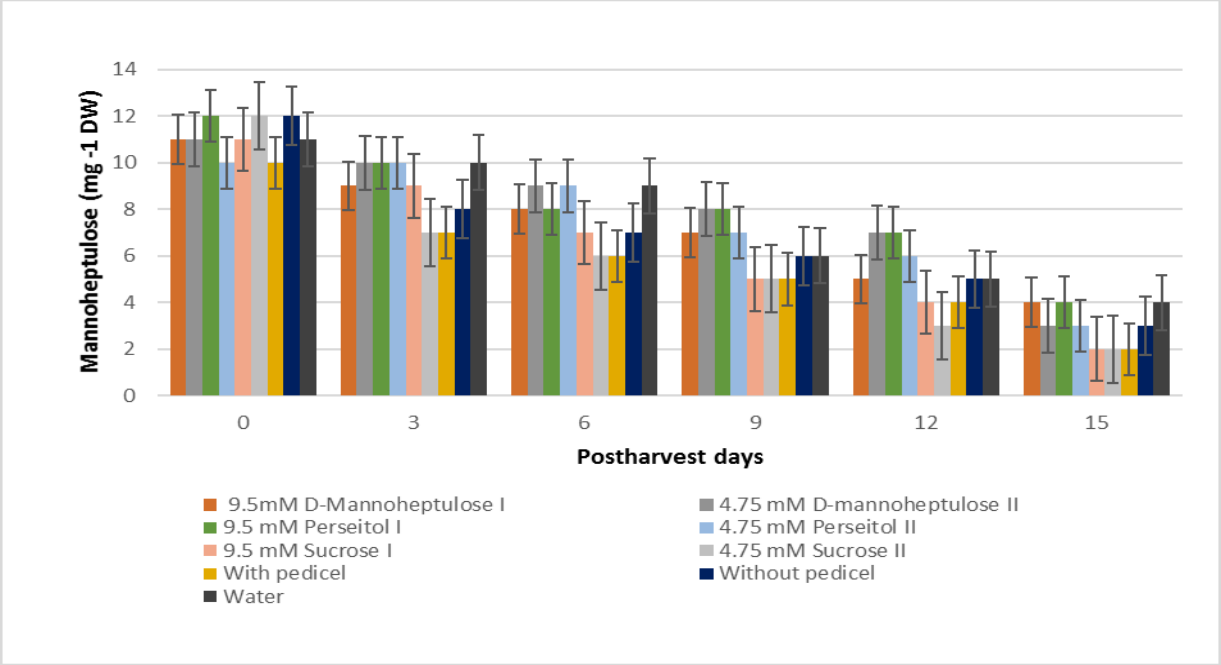

Figure 5: Effect of C7 sugars postharvest infusion treatment, on mesocarp *D*- mannoheptulose concentration of Late-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 12.73

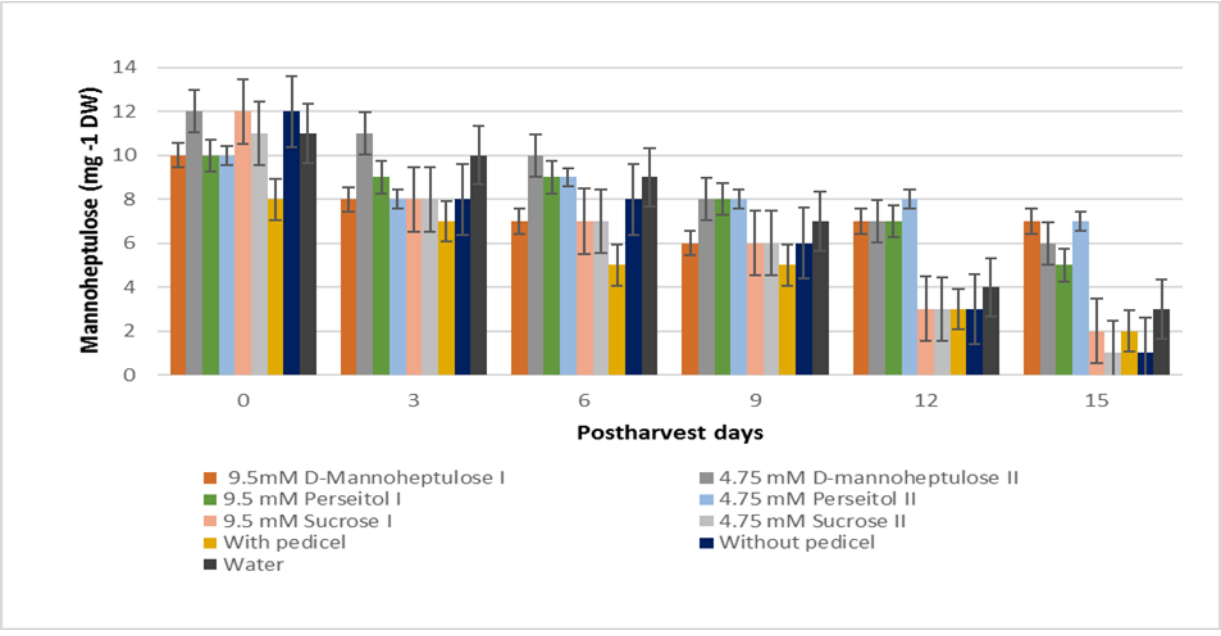

Figure 6: Effect of C7 sugars postharvest infusion treatment, on mesocarp *D*- mannoheptulose concentration of Late-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 13.642

Sugar profile (Perseitol)

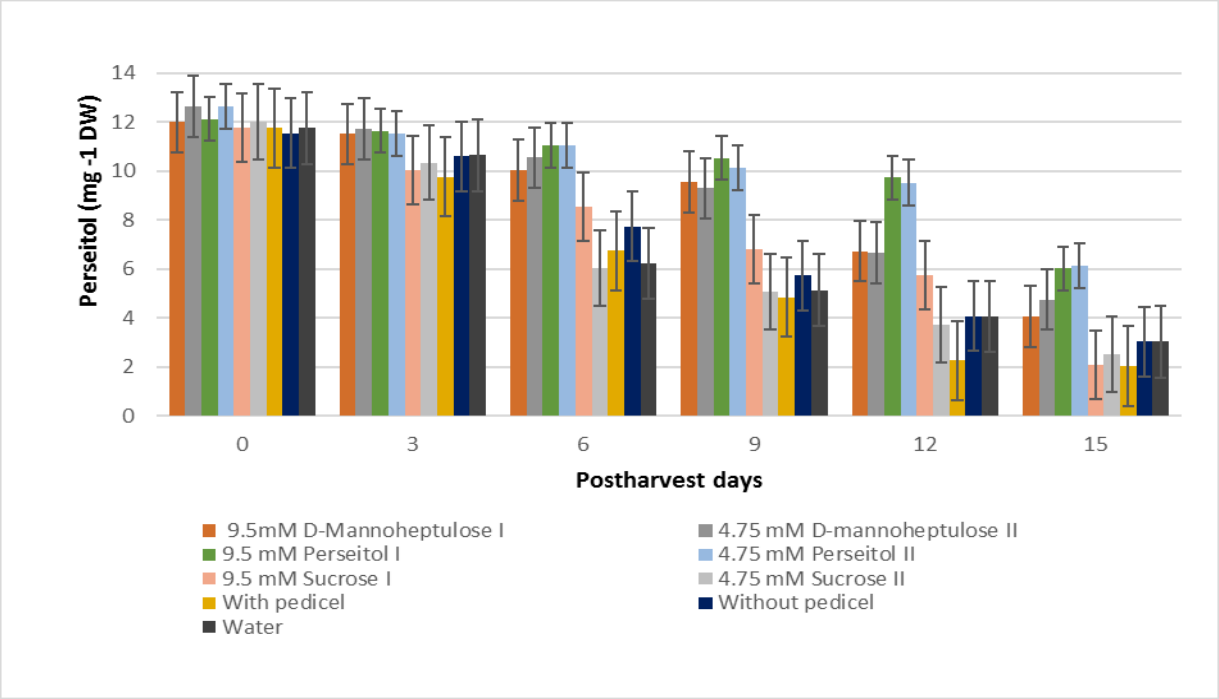

Figure 7: Effect of C7 sugars postharvest infusion treatment, on perseitol mesocarp concentration of Early-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 8.694

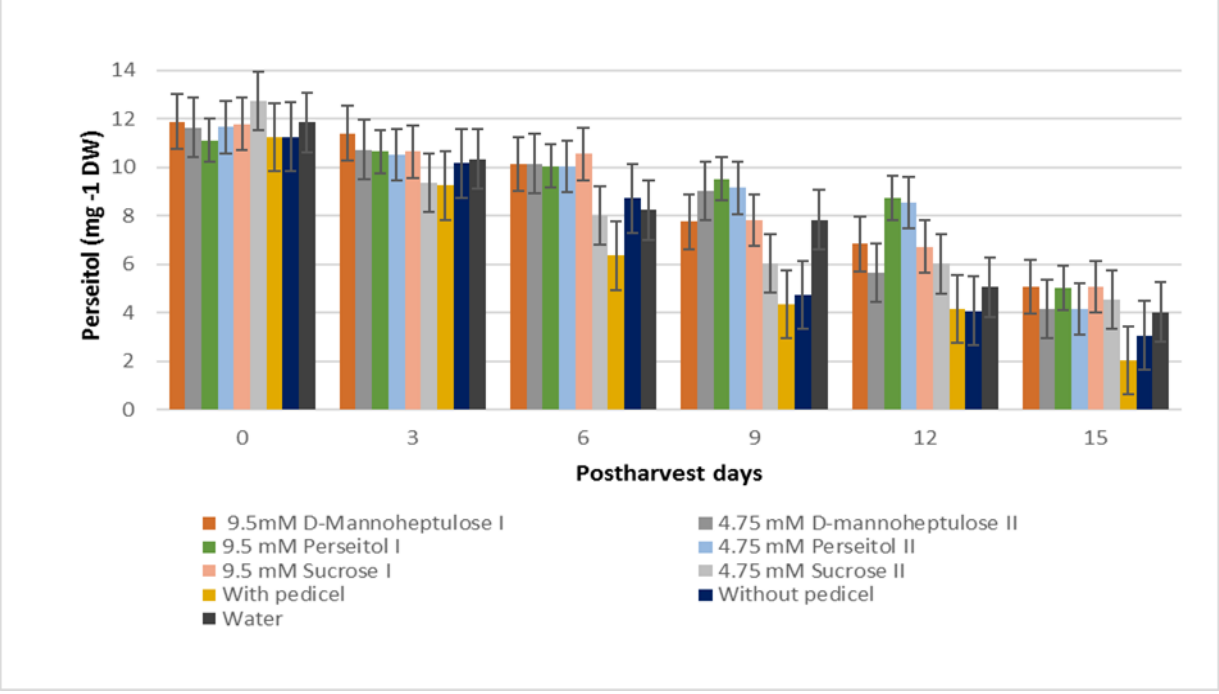

Figure 8: Effect of C7 sugars postharvest infusion treatment, on perseitol mesocarp concentration of Early-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 7.865

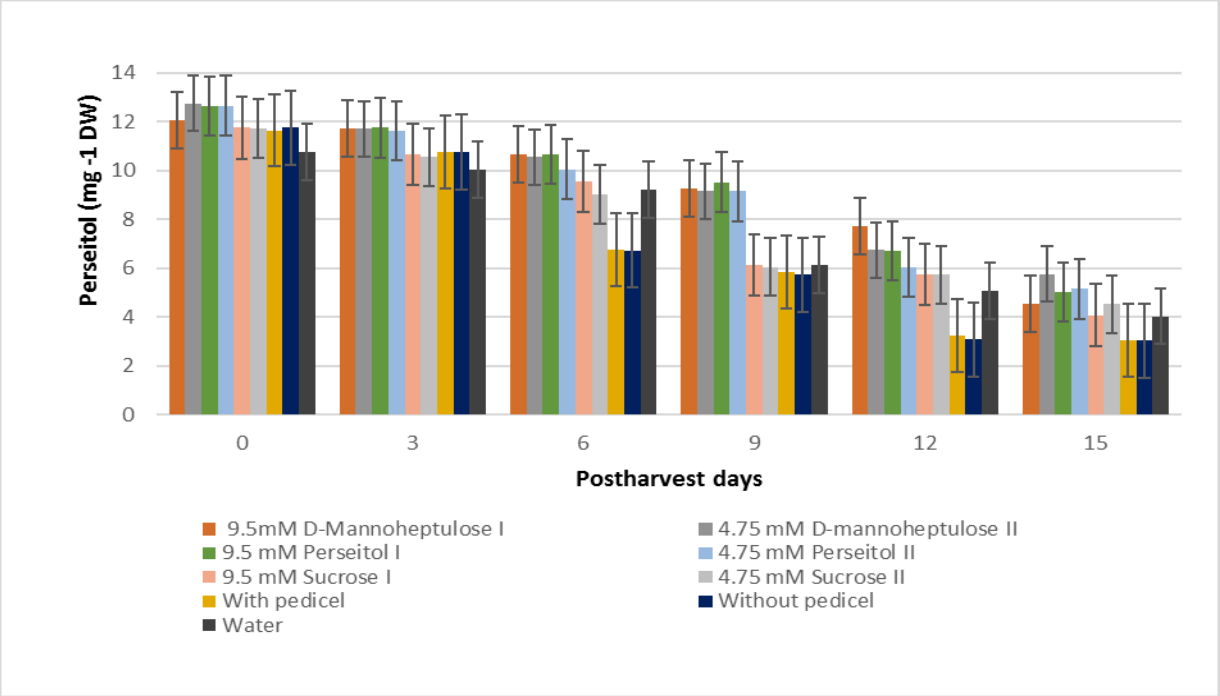

Figure 9: Effect of C7 sugars postharvest infusion treatment, on perseitol mesocarp concentration of Mid-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\,(0.05)}$ = 10.501

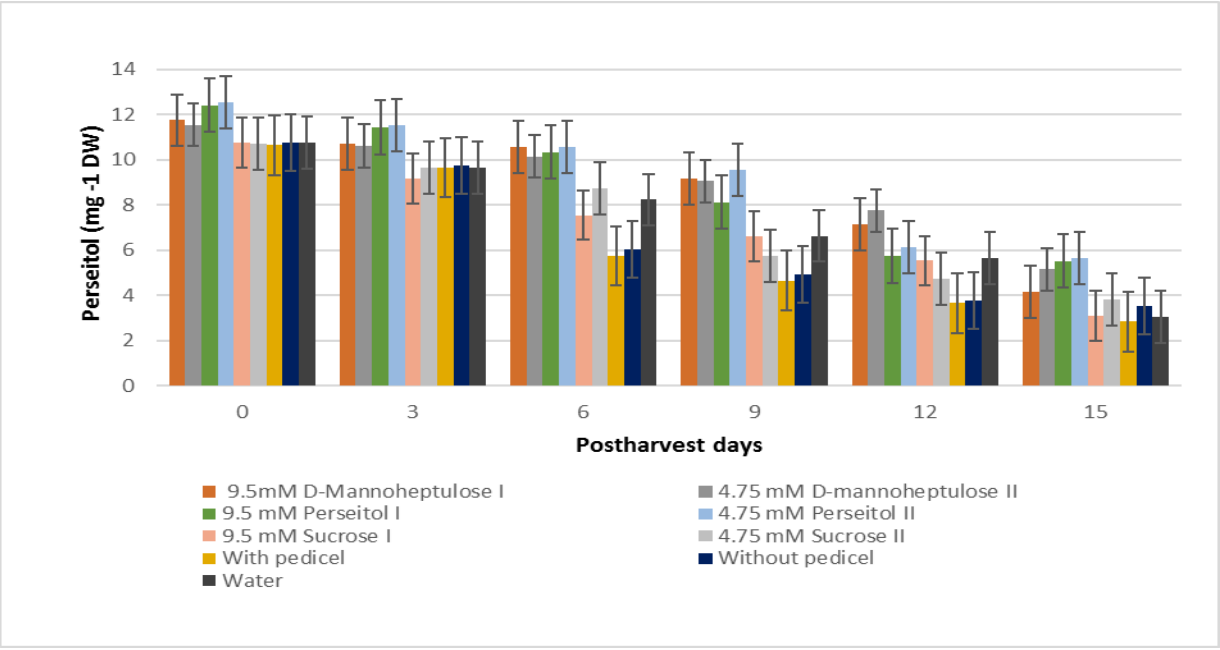

Figure 10: Effect of C7 sugars postharvest infusion treatment, on perseitol mesocarp concentration of Mid-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\,(0.05)}$ = 10.009

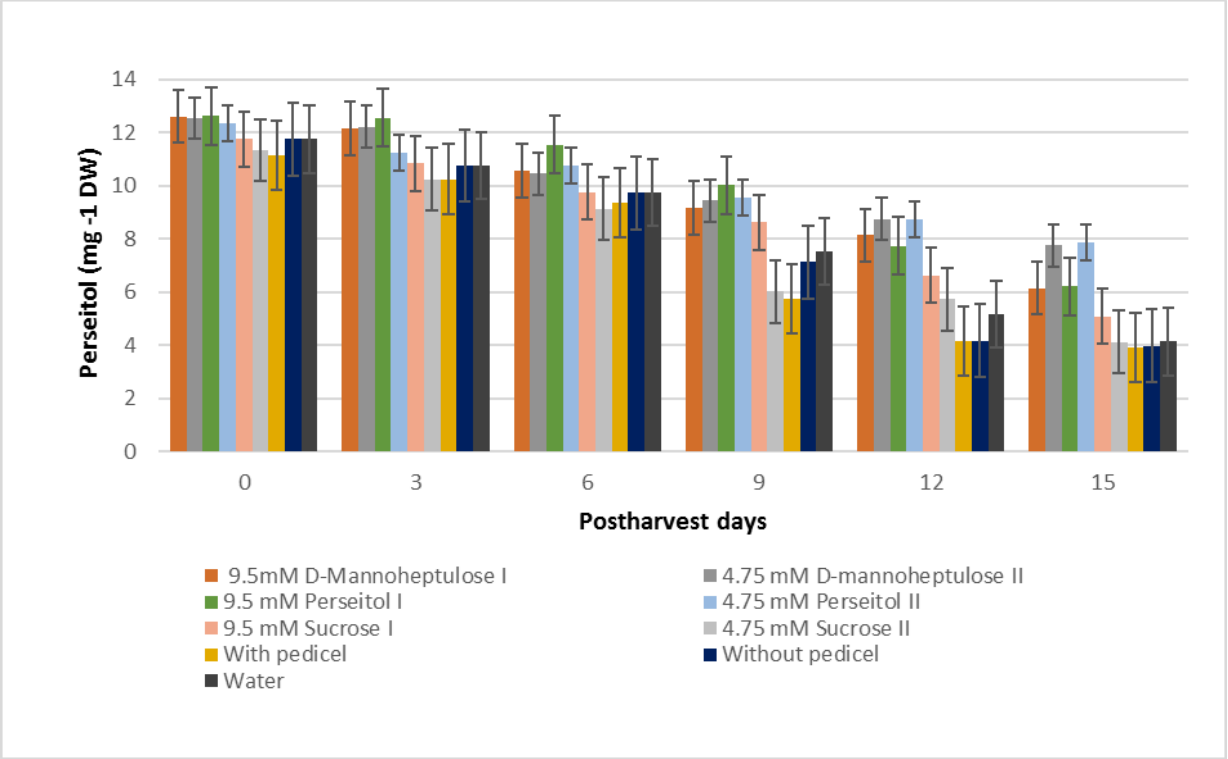

Figure 11: Effect of C7 sugars postharvest infusion treatment, on perseitol mesocarp concentration of Late-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 12.741

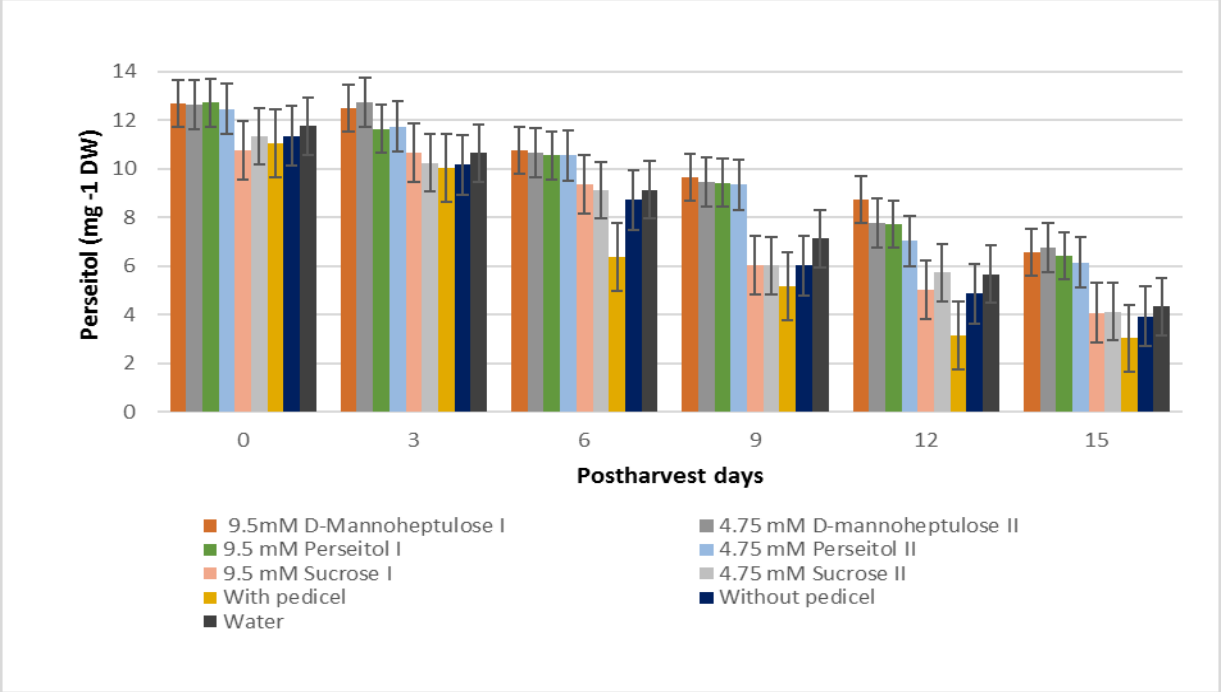

Figure 12: Effect of C7 sugars postharvest infusion treatment, on perseitol mesocarp concentration of Late-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 13.121

Mesocarp moisture content (%MC)

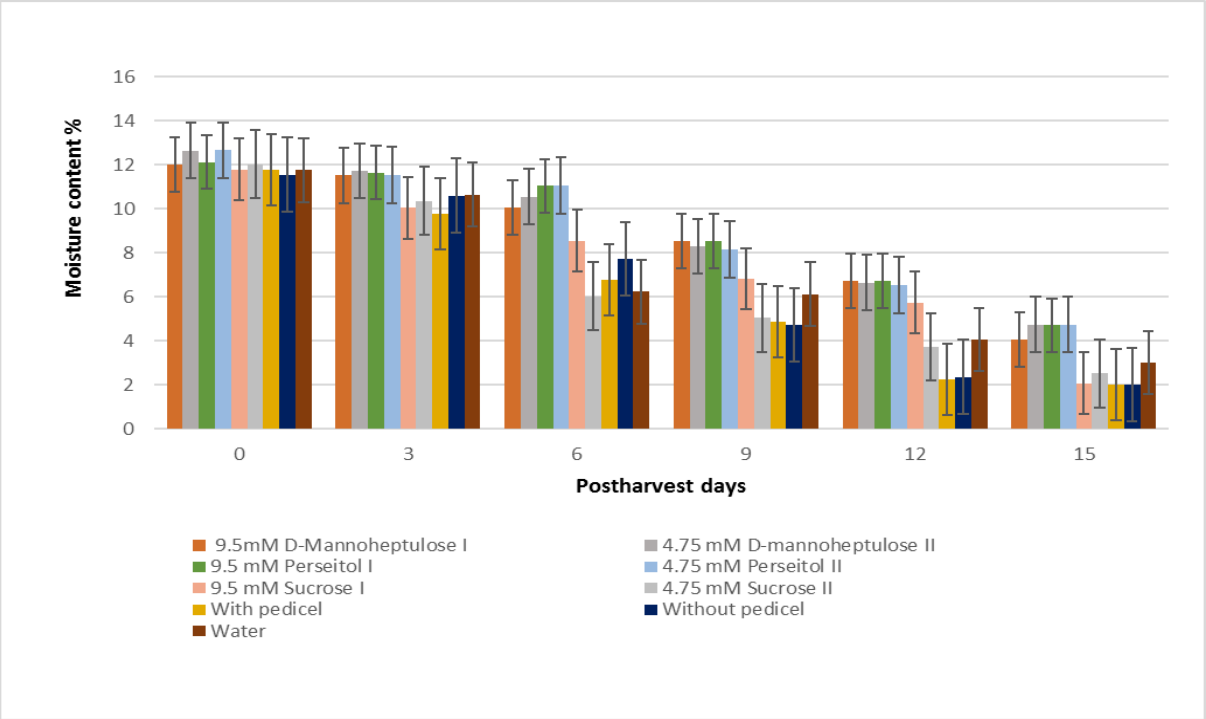

Figure13: Effect of C7 sugars postharvest infusion treatment, on moisture content (MC) of Early-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\,(0.05)}$ = 5.462

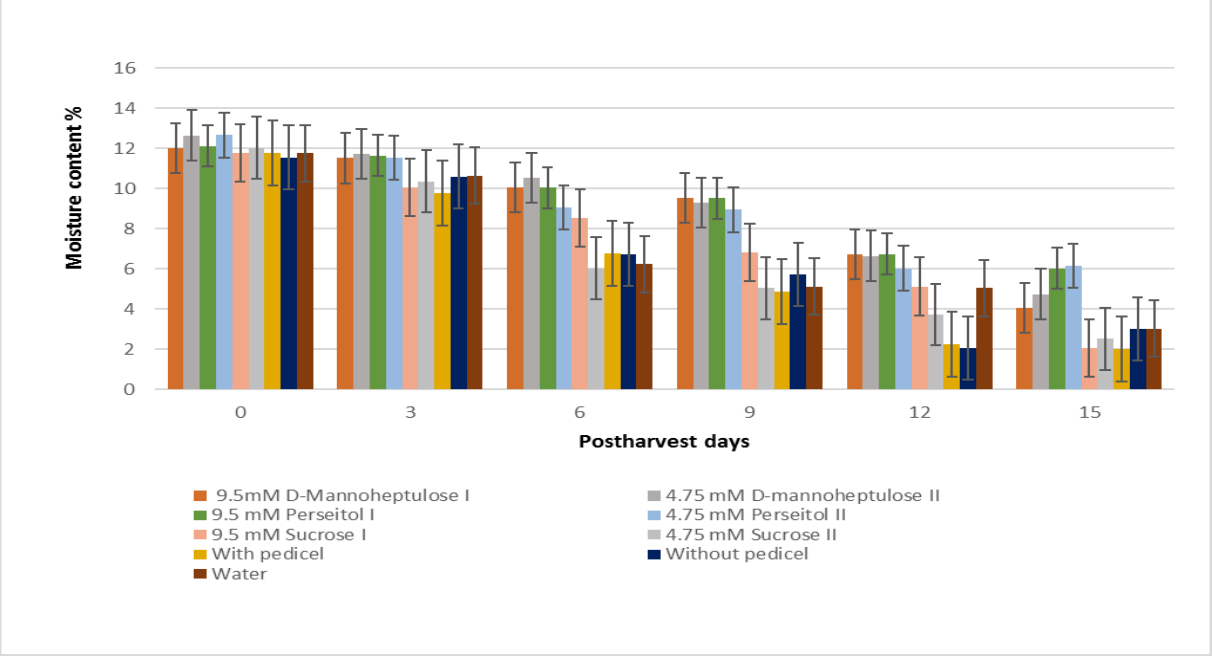

Figure 14: Effect of C7 sugars postharvest infusion treatment, on moisture content (MC) of Early-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\,(0.05)}$ = 6.432

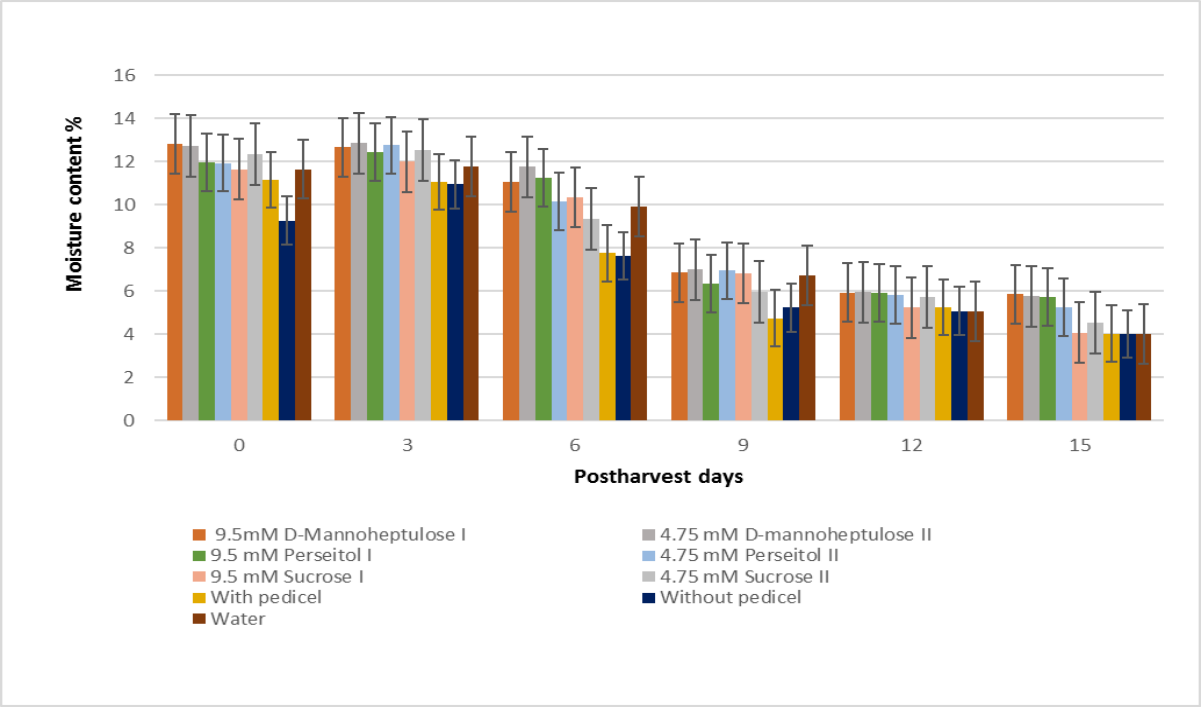

Figure 15: Effect of C7 sugars postharvest infusion treatment, on moisture content (MC) of Mid-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\,(0.05)}$ = 4.662

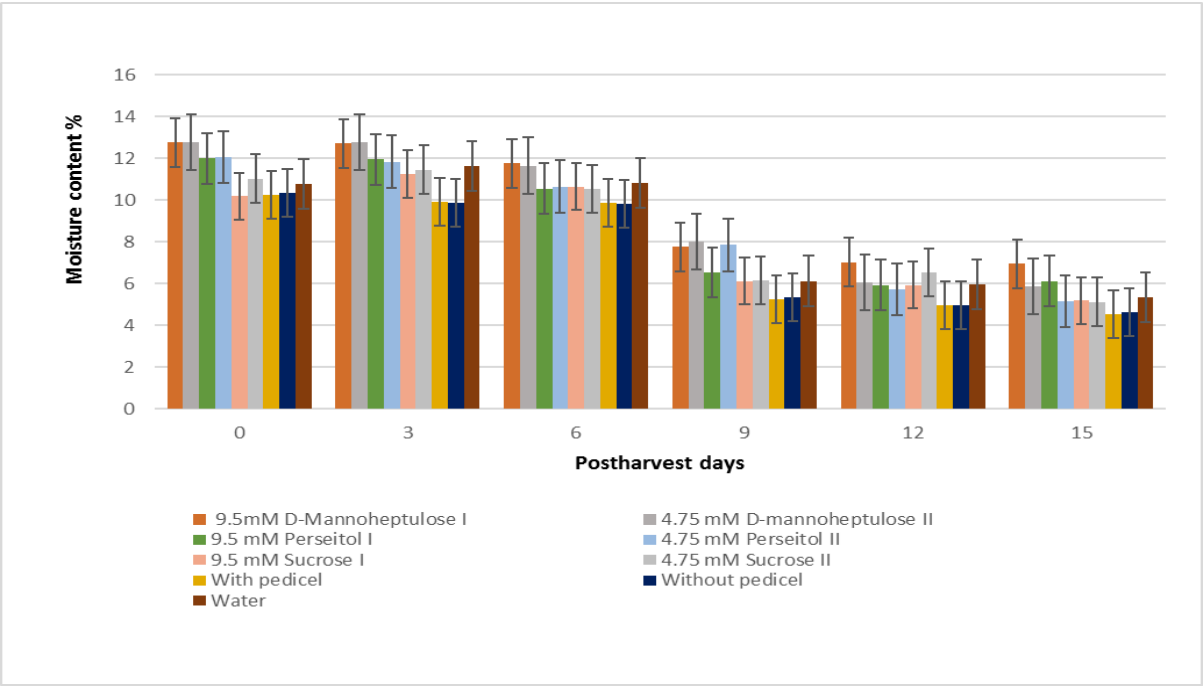

Figure 16: Effect of C7 sugars postharvest infusion treatment, on moisture content (MC) of Mid-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\,(0.05)}$ = 5.167

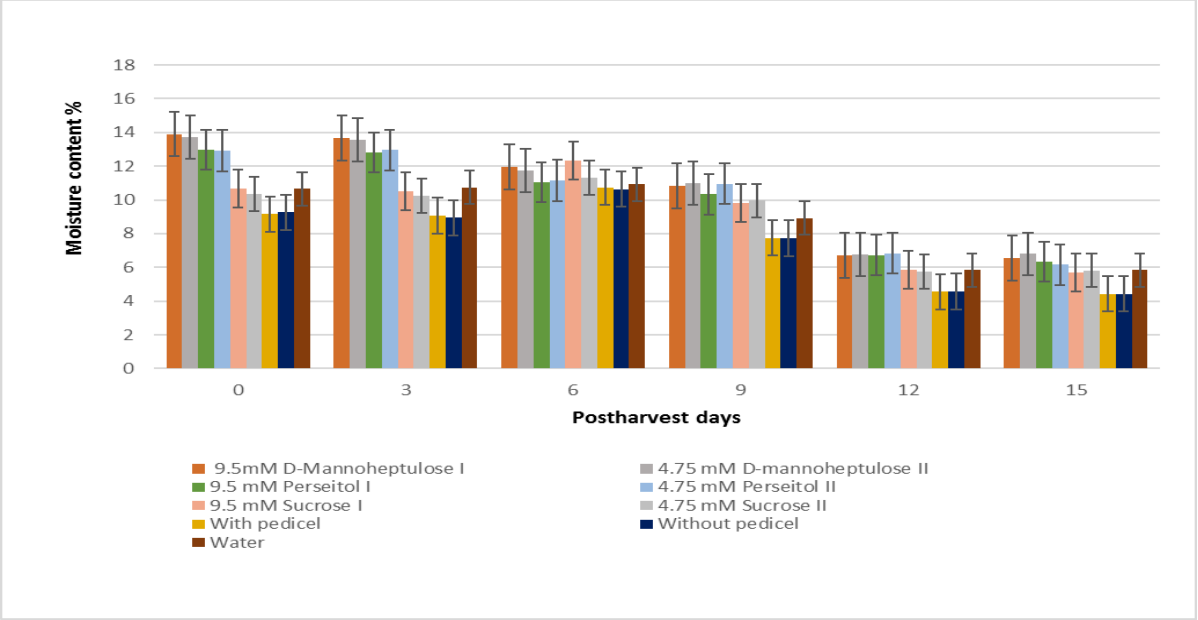

Figure 17: Effect of C7 sugars postharvest infusion treatment, on moisture content (MC) of Late-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 8.960

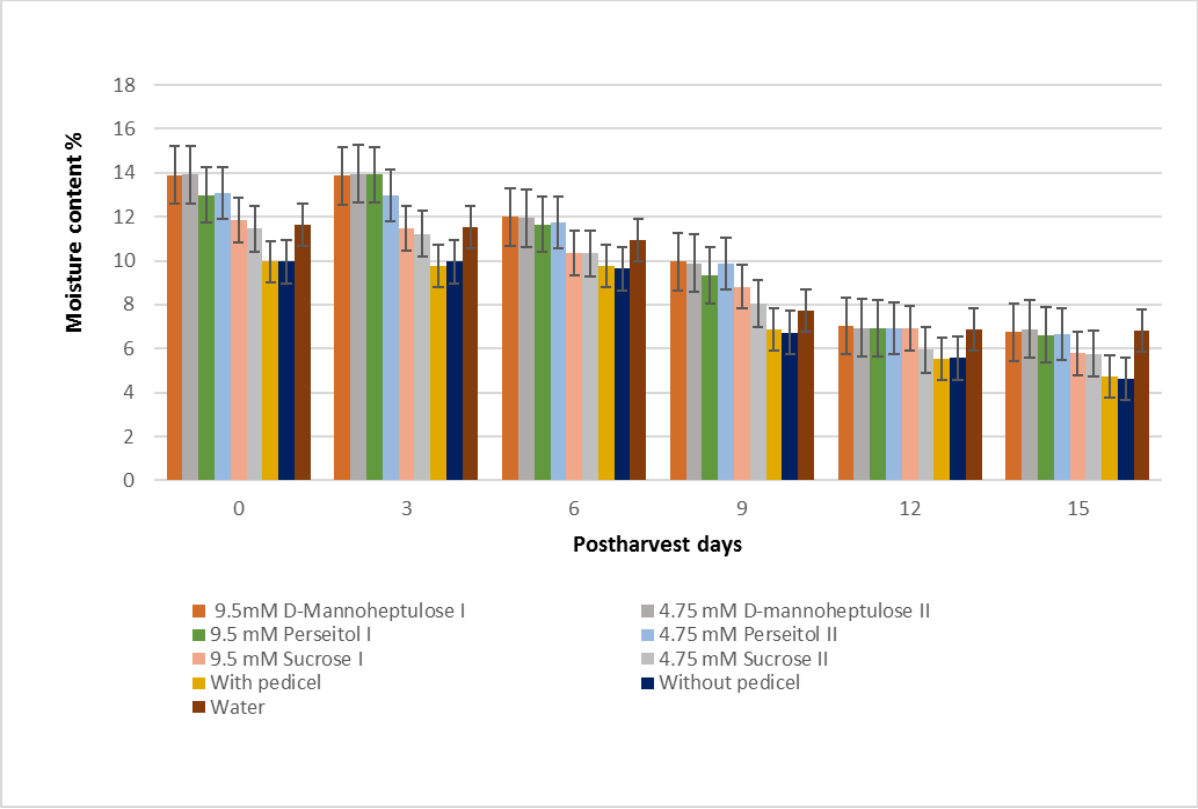

Figure 18: Effect of C7 sugars postharvest infusion treatment, on moisture content (MC) of Late-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 8.002

Dry matter (%DM)

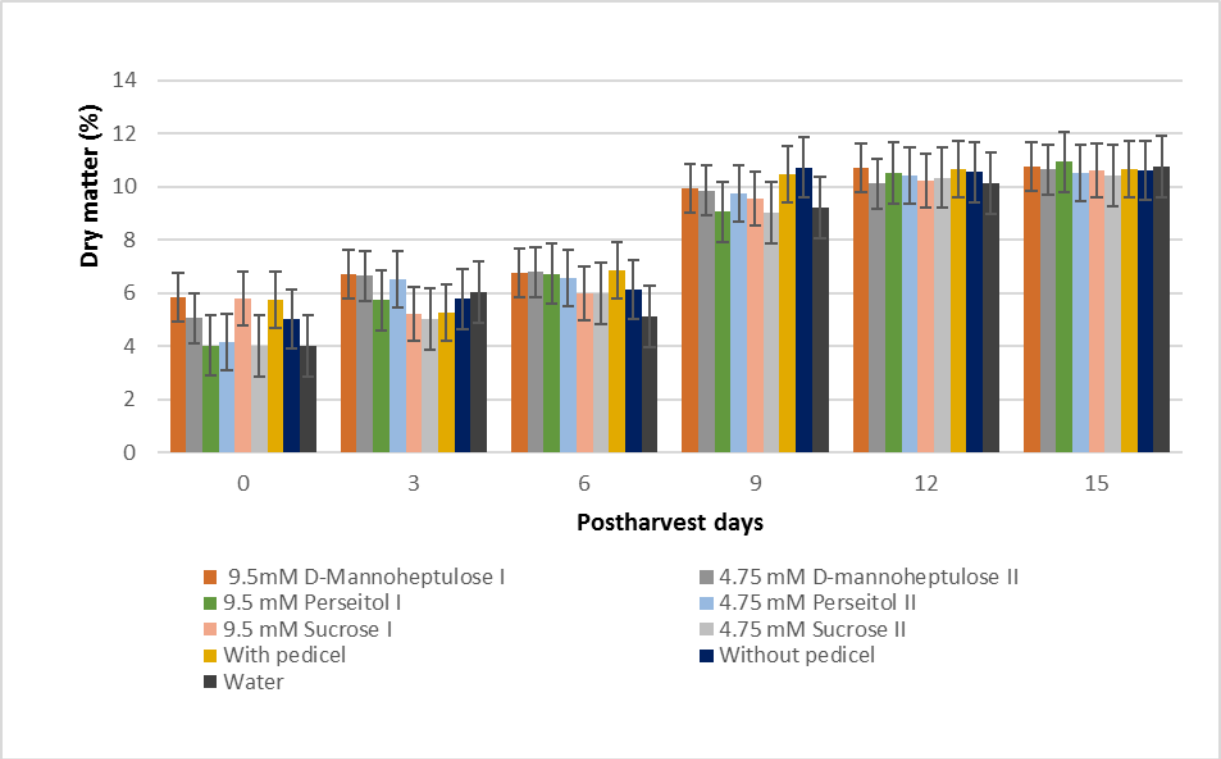

Figure 19: Effect of C7 sugars postharvest infusion treatment, on dry matter (DM) of Early-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 9.807

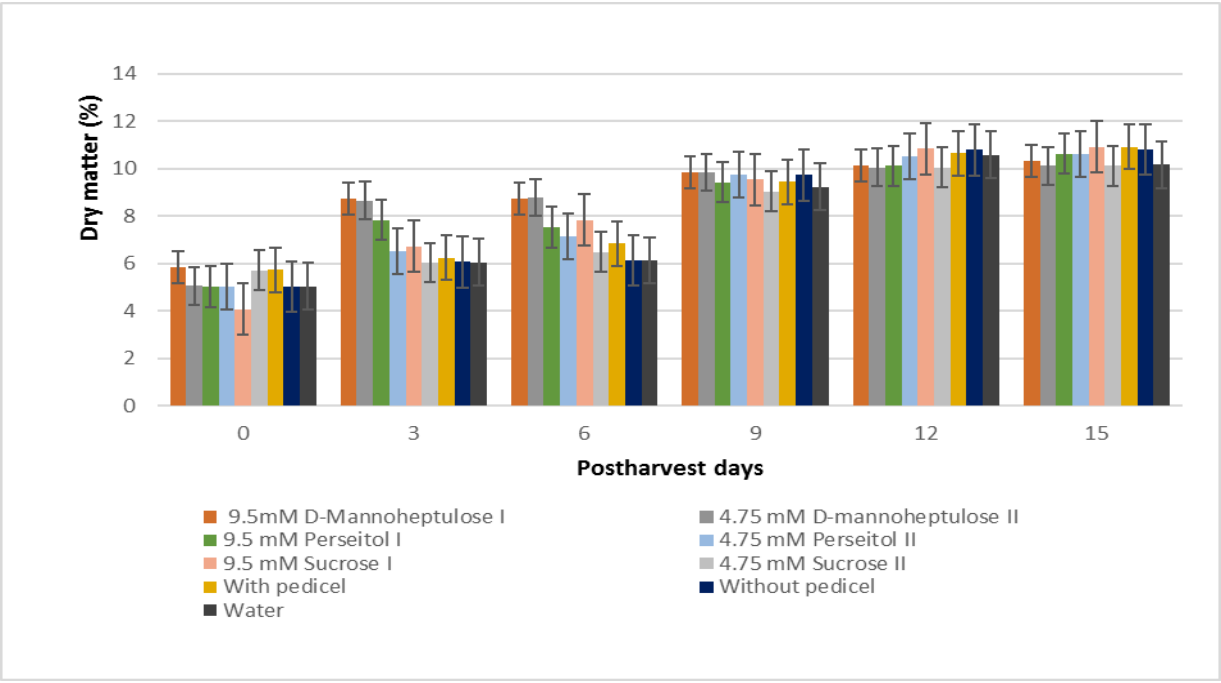

Figure 20: Effect of C7 sugars postharvest infusion treatment, on dry matter (DM) of Early-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 8.103

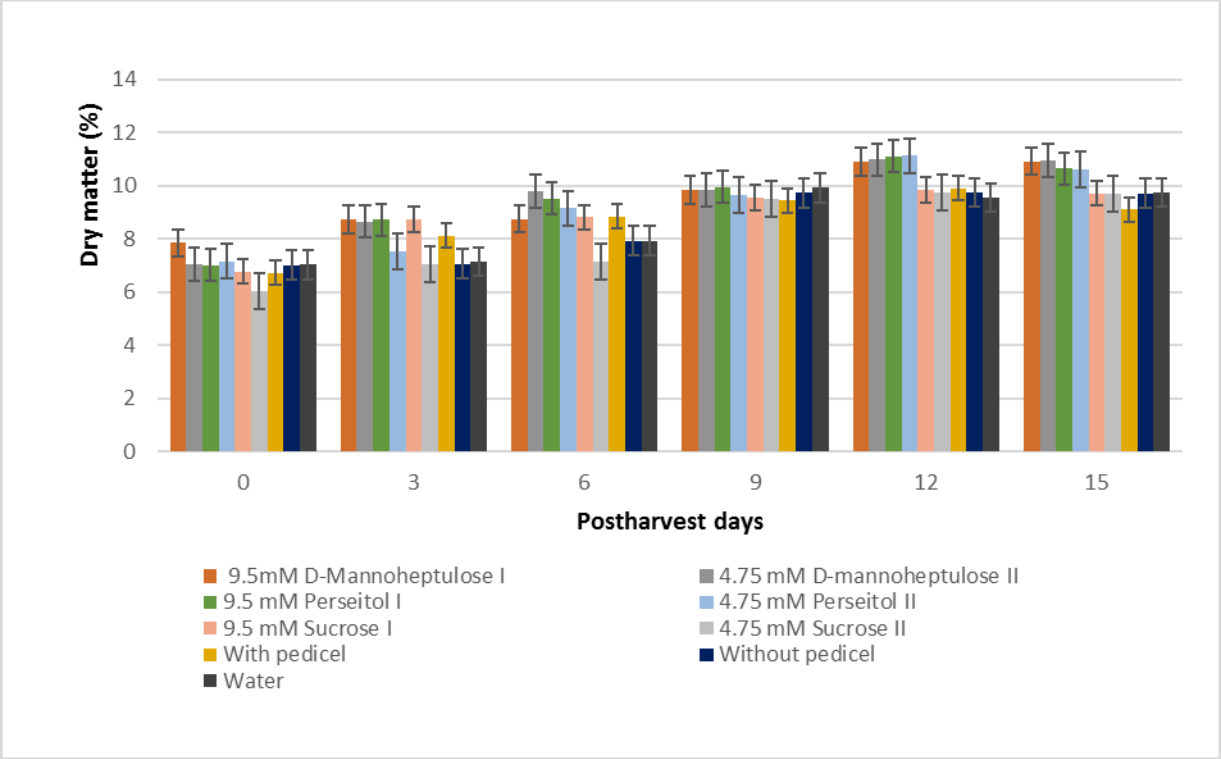

Figure 21: Effect of C7 sugars postharvest infusion treatment, on dry matter (DM) of Mid-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 7.347

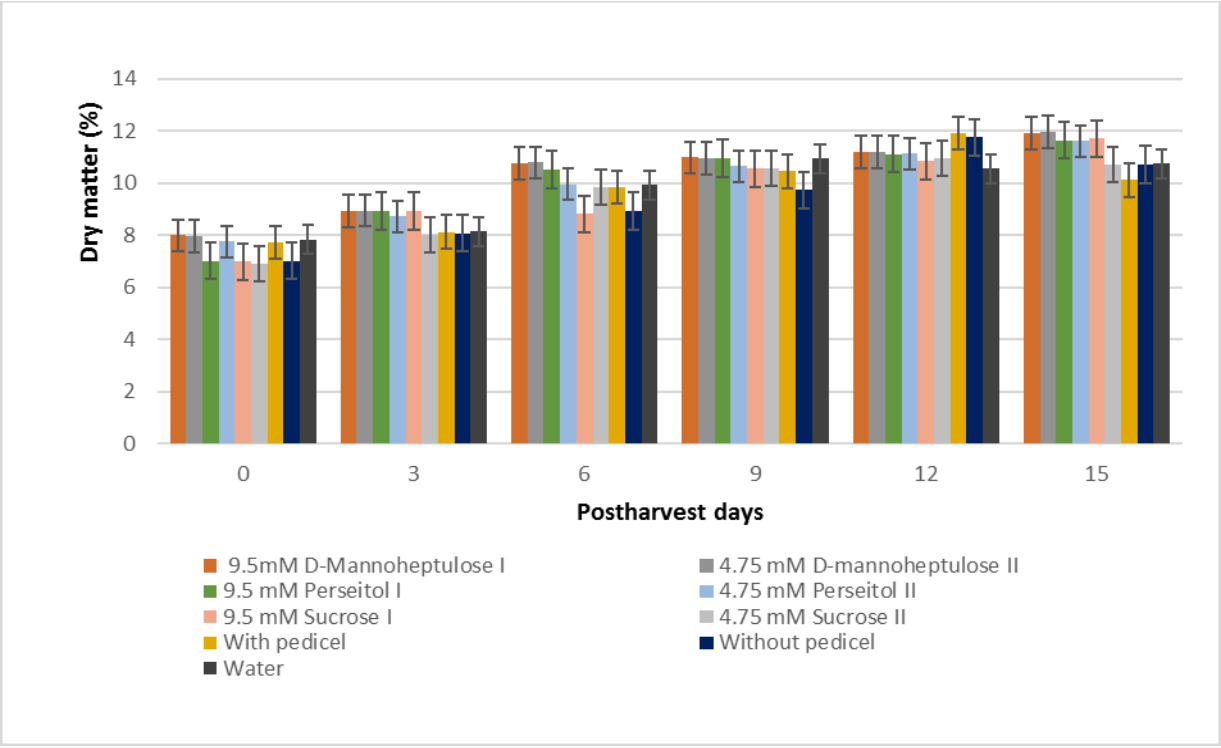

Figure 22: Effect of C7 sugars postharvest infusion treatment, on dry matter (DM) of Mid-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 6.981

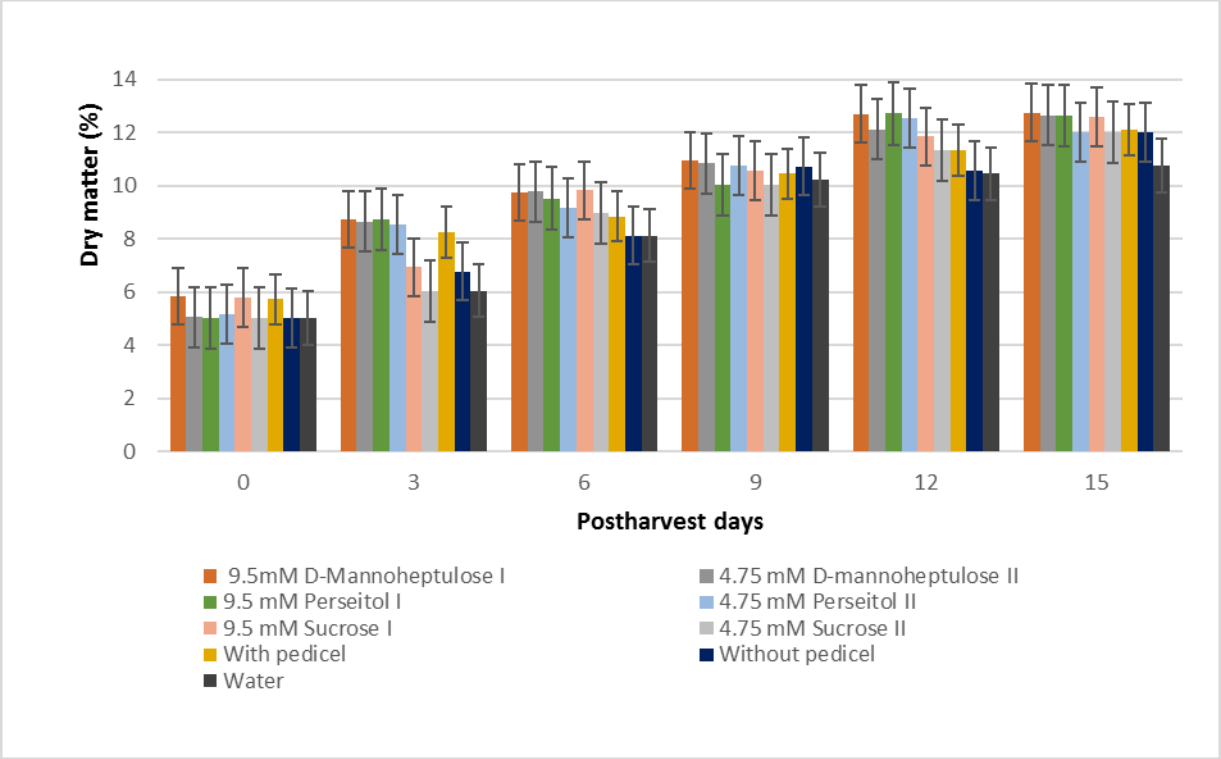

Figure 23: Effect of C7 sugars postharvest infusion treatment, on dry matter (DM) of Late-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 7.615

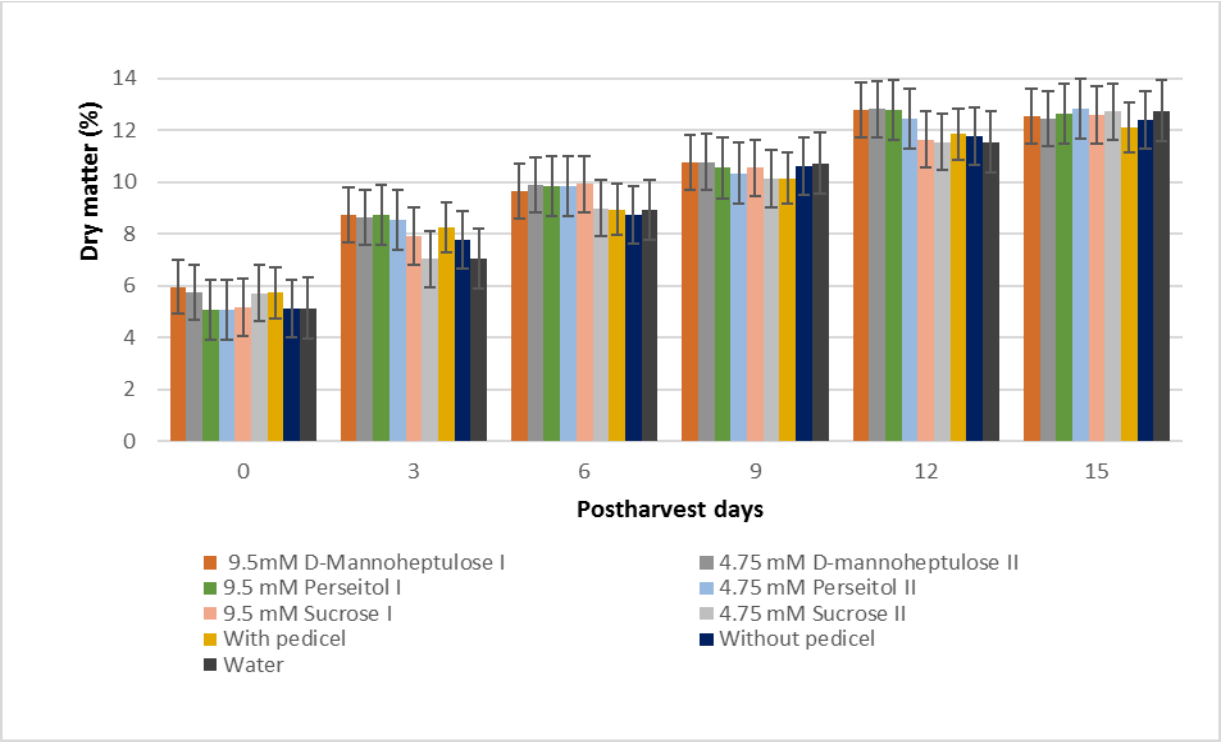

Figure 24: Effect of C7 sugars postharvest infusion treatment, on dry matter (DM) of Late-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 7.534

Mesocarp Oil Content

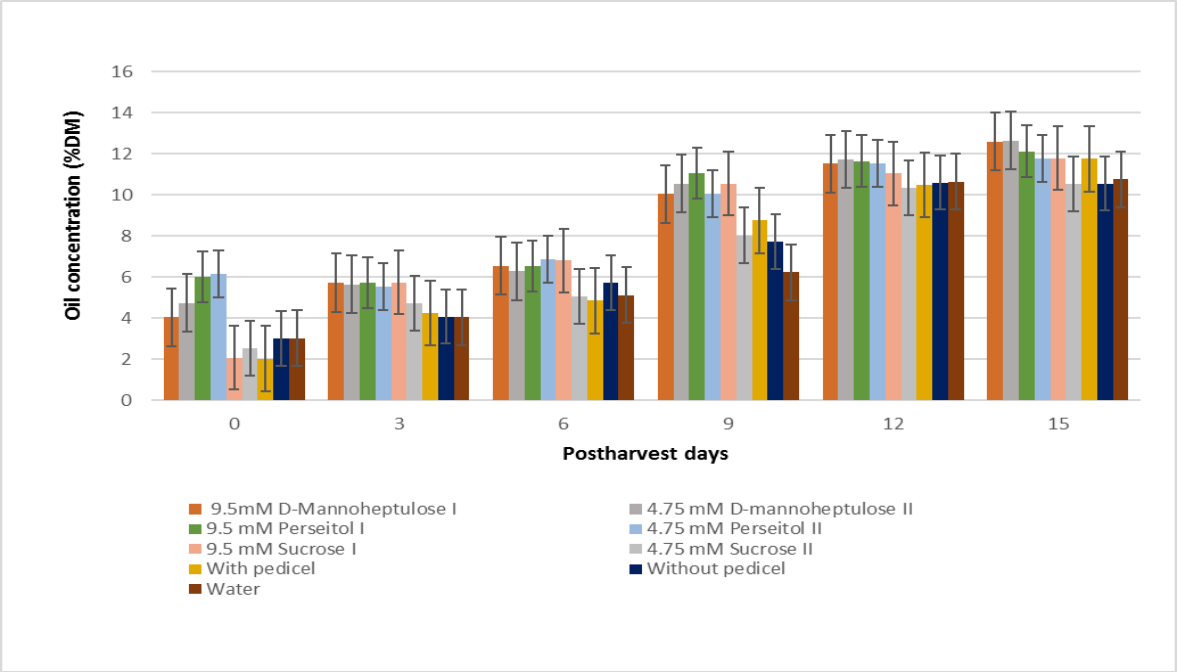

Figure 25: Effect of C7 sugars postharvest infusion treatment, on mesocarp oil concentration of Early-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 10.117

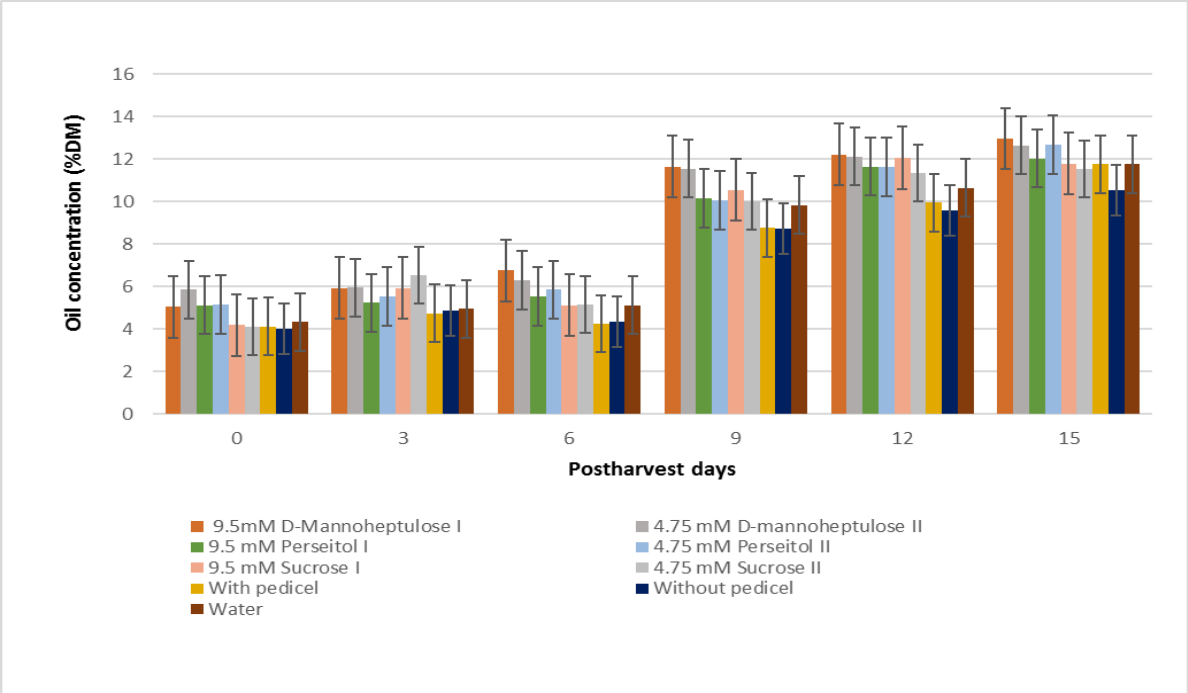

Figure 26: Effect of C7 sugars postharvest infusion treatment, on mesocarp oil concentration of Early-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 12.073

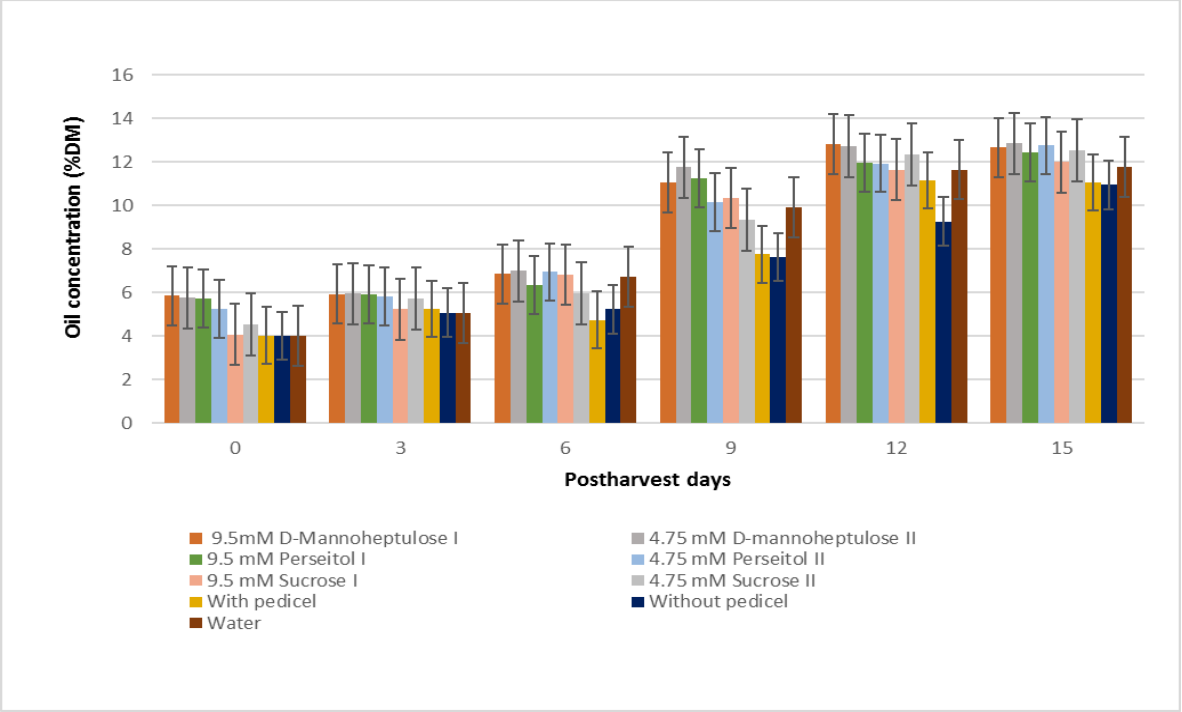

Figure 27: Effect of C7 sugars postharvest infusion treatment, on mesocarp oil concentration of Mid-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 4.6.895

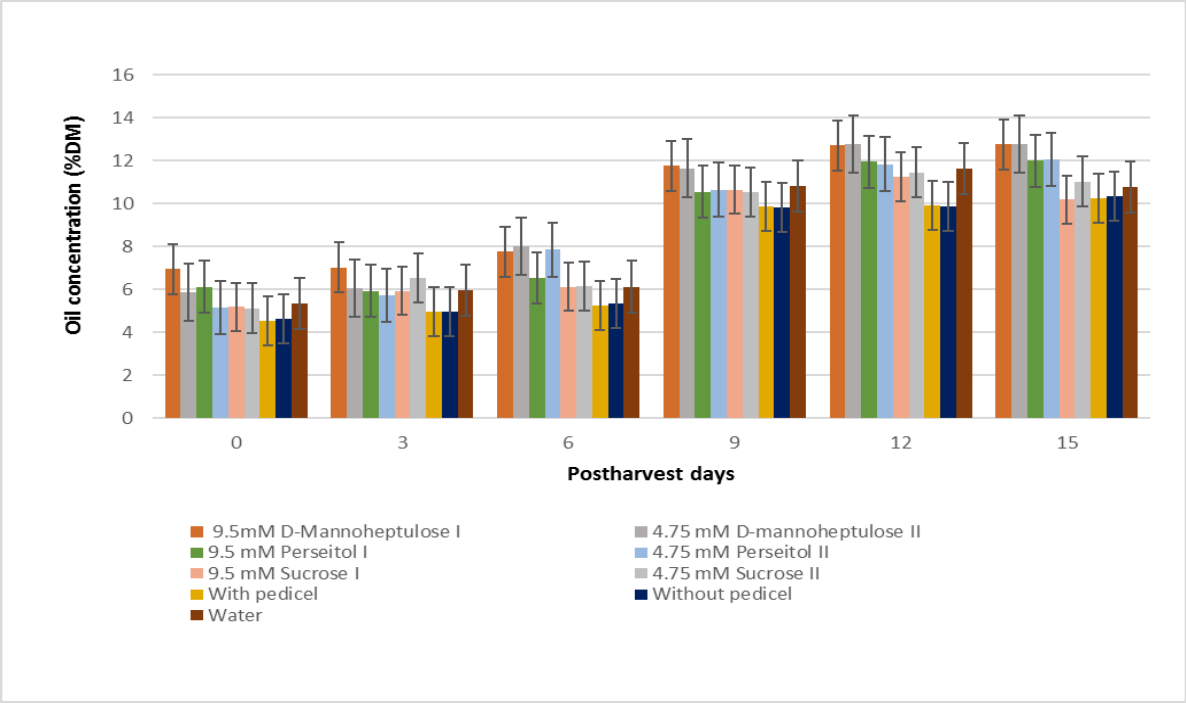

Figure 28: Effect of C7 sugars postharvest infusion treatment, on mesocarp oil concentration of Mid-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 6.842

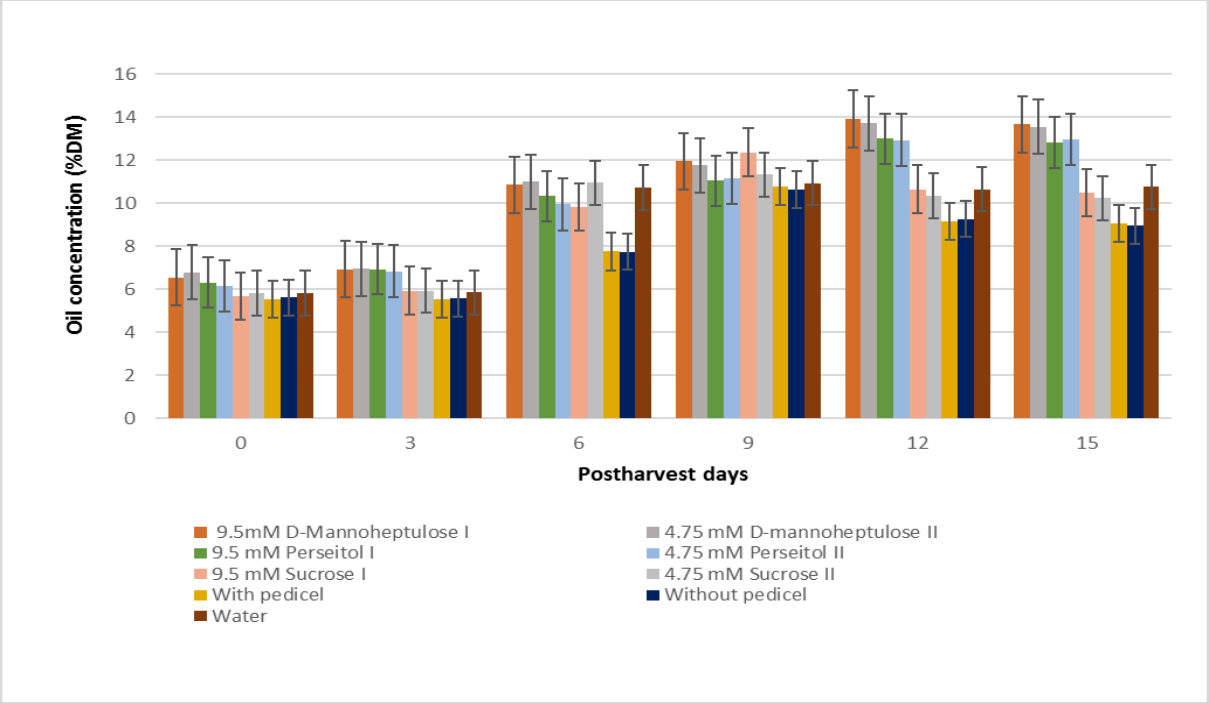

Figure 29: Effect of C7 sugars postharvest infusion treatment, on mesocarp oil concentration of Late-season 'Hass' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 5.410

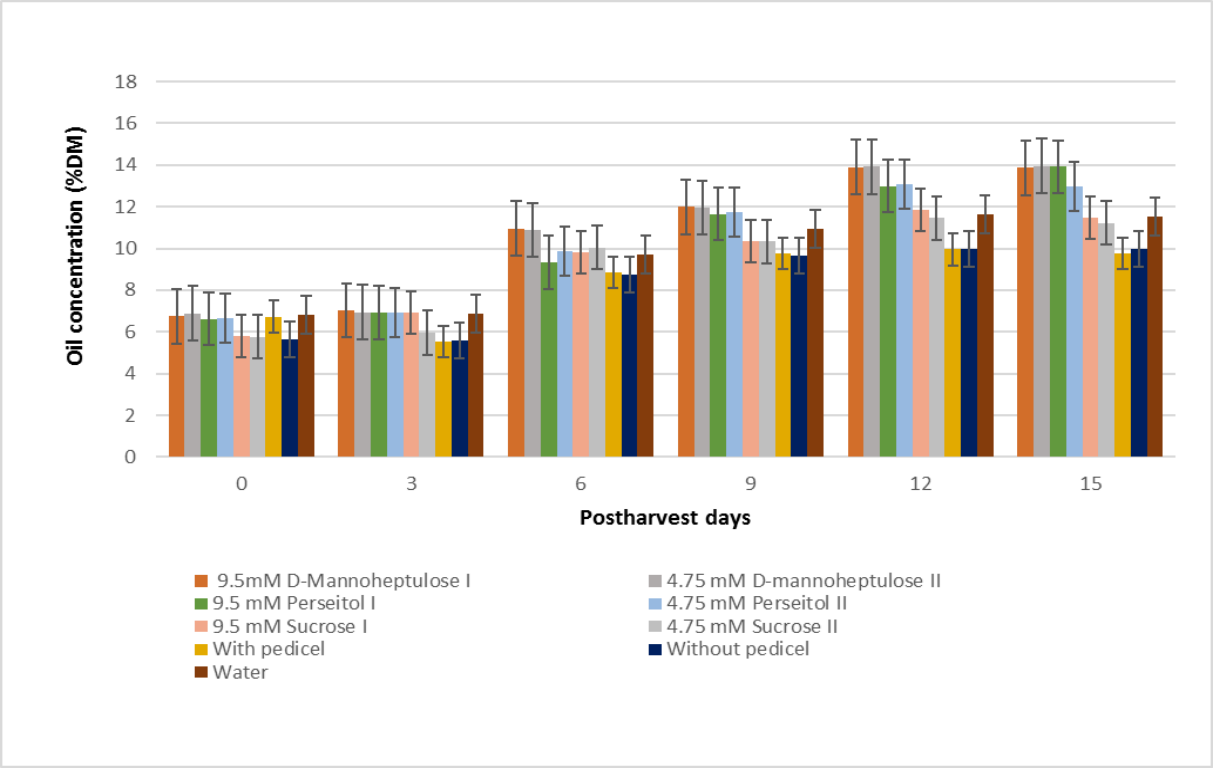

Figure 30: Effect of C7 sugars postharvest infusion treatment, on mesocarp oil concentration of Late-season 'Fuerte' avocado fruit from day of infusion until eat-ripe softness was reached. Vertical bars represent ± SEM. (n=48). LSD $_{(P\ (0.05)}$ = 4.374

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GENERAL DISCUSSION, CONCLUSION AND OUTLOOK

There are many postharvest destructive methods that can be employed to determine compounds associated with fruit maturity. Although C7 sugars have been researched intensively in association with avocado postharvest quality, there are no reports that successfully maintaining C7 sugar pools in the mesocarp tissue can ensure good fruit quality. The overall results of this study suggest that infusion of a watery sugar solution (containing mannoheptulose and perseitol) allow for a better maintenance of the limited pool of C7 sugars found in the mesocarp tissue. This pedicel infusion method significantly reduced the decline in C7 sugar pool. This maintenance of the C7 sugar pools in the mesocarp may be one of the primary reasons for the increase in shelf-life noted under these treatments in a previous study (Bertling and Tesfay, 2011). Perseitol concentrations did not significantly decrease during the early days of postharvest infusion, but did decrease significantly during ripening, which concurs with Liu *et al*. (1999). Thus, as more energy is needed during ripening, it appears that mannoheptulose is utilised during storage and then perseitol is converted to mannoheptulose when the pool has been depleted. Thus, at the end of the infusion period by the time measurements were taken, mannoheptulose concentrations were low and had a minimal effect on fruit quality. There is an importance to build up the C7 sugar pool during fruit growth and development, so that the fruit can be sustained during postharvest at high quality, as this research has shown that keeping the C7 sugar pool high, extends shelf life and fruit quality. This study has therefore, contributed to the understanding of avocado postharvest physiology and should aid in better management of avocados for improved fruit quality and consumer satisfaction.

FUTURE RESEARCH AND COMMERCIAL IMPLICATION

The C7 sugars mannoheptulose and perseitol could be used as bio-markers of inherent quality characteristics of avocado fruit; therefore, postharvest losses of avocado fruit could be minimised, if levels of these sugars can be maintained. It has been shown that C7 sugar and fruit water content can influence ripening physiology. While an infusion method to balance or maintain fruit water and mesocarp C7 sugars content within a consignment is not possible commercially, the experiments have clearly demonstrated that the maintenance of a certain size C7 sugar pool is of importance to manage fruit quality postharvest, as it was demonstrated that the levels of C7 sugars and water in the mesocarp influence ripening. In South Africa, some packhouses use a wet dump, whereby the fruit are dumped into a water bath usually containing a fungicide. In such a wet dump technique, fruit may take up some water, particularly laterally towards the seed (Lee *et al.*, 2006), thereby allowing for a higher mesocarp moisture content that delays ripening.

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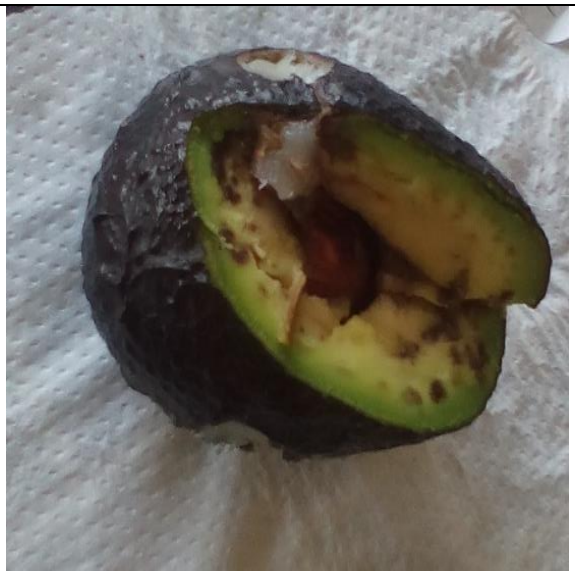
APPENDIX

A (Vascular browning of late-harvested Fuerte)



B (Vascular browning of late-harvested Hass)

C (Rubbery mesocarp of early-harvested Fuerte)

D (Shrinkage of early-harvested Hass)

Figure A1: Internal effect of sugars infusion, on (Vascular browning: Fuerte A and Hass B); (mesocarp rubbery: C, and exocarp shriveling: D) in different harvesting seasons (early, middle, and late season) **(Chapter 2).**