EVALUATION OF MATURITY PARAMETERS OF ‘FUERTE’ AND ‘HASS’ AVOCADO FRUIT

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

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DEDICATION

This thesis is dedicated to my parents, Mr and Mrs Olarewaju, my siblings, Olaide and Timilehin Olarewaju, my alter ego, Johnson Oluwadele and my sweetheart, Olufunke Fajinmi.

PREFACE

This thesis is a compilation of manuscripts where individual chapter is an independent article introduced disjointedly. Hence, some repetition between individual chapters has been inevitable. Each chapter in this thesis is formatted to the requirements of Elsevier BV Publishers of Postharvest Biology and Technology.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

OVERVIEW

Avocado fruit is one of the most important horticultural crops produced in South Africa. The fruit does not give obvious indication of maturity as it does not ripen as long as it remains attached to a tree. Harvesting avocado fruit at full physiological maturity, a stage at which it will continue normal development, plays a vital role in the postharvest physiological processes and the successful postharvest management of the fruit. Common maturity parameters used in various avocado fruit industries include mesocarp oil content, moisture content (MC) and dry matter (DM). However, the difficulty of measurement (oil content) and unreliability (MC and DM), can result in immature fruit reaching the consumer. To ensure that avocado fruit of good quality are delivered to the market and for growers to maximise profits, possible factors indicating optimal harvest maturity were investigated during the South African 2013 and 2014 avocado growing season. Additionally, the growth pattern of fruit, beyond what is currently regarded as physiological maturity, was examined for the possibility of the fruit exhibiting a double sigmoidal growth pattern, typical of nut crops. Fruit were harvested from two commercial orchards in the cool subtropical area of KwaZulu-Natal, South Africa. Fruit were harvested bi-weekly from February to March and then monthly from April to October, 2014. The MC decreased over the harvesting period, ($p < 0.001$), while oil content increased ($p < 0.001$). The study of the pattern of avocado fruit growth and development over the eight months observation period revealed that the fruit exhibits a single sigmoidal growth pattern. It could also be deduced from the experimental results that MC is a better indicator of maturity compared with oil content.

In a quest to find an alternative maturity indicator that could provide a more reliable measure of avocado harvest maturity, total soluble solids (TSS) was evaluated for the possibility of providing an objective maturity index. Seven carbon (C7) sugars, *D*-mannoheptulose and perseitol, are dominant sugars in avocado fruit and have been suggested as likely indicators of avocado fruit maturity. *D*-mannoheptulose, a major component of mesocarp TSS, has been suspected to be responsible for the continued growth of the fruit. 'Fuerte’ and ‘Hass’ avocado fruit were harvested during the early, mid and late harvesting period in 2013 from Bounty Farm and during the 2014 season (February to August) from Bounty Farm and Everdon Estate. Sample ws taken along the equatorial region of each fruit and analysed for TSS, measured by squeezing juice out of the mesocarp using a garlic press and determining its °Brix using a digital refractometer. A high level of significant difference was observed between TSS and harvesting period for ‘Fuerte’ during both seasons ($p < 0.001$) and a significant difference was found between the two production locations during the 2014 growing period ($p < 0.001$). There was no significant difference ($p = 0.344$) between production sites for ‘Hass’ fruit harvested during the 2014 season. The results of the study reveal that TSS cannot be used as an indicator of avocado fruit maturity.

In an attempt to non-destructively predict maturity parameters of avocado fruit, a total of 150 intact avocado fruit were scanned in reflectance mode of near-infrared spectroscopy (NIRS) during the 2013 and 2014 growing seasons. Reference maturity parameters, including MC, DM and oil content were measured using conventional destructive methods. Calibration models developed during 2013 season were used to predict the dataset acquired during 2014. NIRS prediction results showed that MC and DM were predicted with significant accuracy compared with oil content, prediction of which was not accurate. The prediction statistics for NIRS predicted MC and DM content demonstrated the potential of this system for non-destructive evaluation of avocado fruit maturity parameters (MC and DM). The high prediction accuracy recorded when models developed in 2013 were used to predict maturity of fruit harvested during the 2014 season demonstrated robustness of partial least square (PLS) models. Where speed and accuracy are required for assessing the maturity status of individual, intact avocado fruit, the method developed in this study is recommended.

CHAPTER 1

GENERAL INTRODUCTION AND RESEARCH AIM

1. Introduction

Avocado fruit (*Persea americana* Miller) is one of the major economically important subtropical fruit crops grown in South Africa. According to a report by NAMC (2013) of South Africa, about 45% of 110 000 t of avocado produced annually are exported. Globally, the South African avocado industry is ranked the seventh largest exporter of avocado industries worth over R319 million rand ($30 million) in 2011 (FAOSTAT, 2014). It is difficult to determine avocado fruit maturity and to predict its ripening patterns, particularly due to its climacteric ripening pattern of physiology (Lee et al., 1983). Dhatt and Mahajan (2007) reported that fruit maturity is one of the major factors to be considered before harvesting fruit as it dictates the postharvest quality of such fruit. Therefore, ensuring that fruit have reached full physiological maturity at the time of harvesting plays a vital role in its eating quality and determines how the fruit can be handled, stored, and transported (Reid, 1985). Thus, in-depth knowledge of fruit maturity and its parameters is essential to deliver avocado fruit of excellent quality to the consumers.

Avocado fruit is considered physiologically mature when most of its natural growth and development has occurred, while commercial maturity is defined as the phase of development at which harvested fruit will undergo the standard process of ripening and eventually provide satisfactory eating quality (Lee et al., 1983). In other words, a physiologically matured avocado fruit can be considered as commercially matured. Harvesting immature avocado fruit can result in economic losses due to poor fruit quality once the fruit has ripened. Symptoms of poor quality resulting from harvesting immature avocado fruit include shrivelling, rubbery texture, stringy vascular tissue, insipid flavour and increased rots (Lee et al., 1983; Kader, 1999; Pak et al., 2003). Over-mature fruit on the other hand, are oily and can turn rancid soon after harvest (Kader, 1999). A fruit harvested either premature or over-mature are more vulnerable to postharvest physiological disorders than fruits harvested at the proper maturity state (Kader, 1999). Consequently, to determine the stage of development at which a fruit meets minimum acceptable quality, studies that will identify quicker, easier and more reliable techniques measuring maturity of individual avocado fruit is therefore crucial for the advancement of avocado industry.

Several authors have recommended the use of oil content as a parameter for determining maturity of avocado fruit (Young and Lee, 1978; Lee et al., 1983; Mizrach and Flitsanov, 1999; Hofman et al., 2002); however, due to the tedious, time consuming, and expensive nature of determining oil content other parameters, such as mesocarp moisture content (MC) or its reciprocal, dry matter (DM), are currently used. (Hofman et al., 2002; Blakey, 2011). Previous studies also documented that the dominant sugars in avocado, D-mannoheptulose and perseitol, play major roles as determinants of postharvest fruit quality (Liu et al., 2002; Bertling and Bower, 2005; Bertling et al., 2007). Determination of total soluble solids (TSS) is a quicker and less expensive maturity parameter to measure compared to oil content and DM. Carbohydrates, fats, proteins and minerals of many fruit comprise the TSS of fruit (http://www.yara.us/agriculture/crops/citrus/crop-nutrition/citrus-quality/total-soluble-solids/). The TSS content of a fruit is a simple and easily accessible determinant of internal quality of many fruit which represents soluble sugar and acids or solids in a given fruit (Eshed and Zamir, 1995). This quality parameter has been reported as a reliable determinant of quality in citrus (Stevens and Baier, 1939), apple (Drogoudi et al., 2008), and tomato (Eshed and Zamir, 1995). Furthermore, TSS is commonly measured to give an overview of grape maturity at harvest (CRV, 2005); however, its use as possible maturity determinant of avocado fruit has not been evaluated.

Currently, oil content is considered the most reliable parameter for determining avocado fruit maturity (Kaiser, 1994), but is not commercially used by all avocado industries because of the difficulty, time consuming and expensive nature of measurement. Instead, MC or its reciprocal value, DM, is used because it is easier to determine but is not very reliable (Hofman et al., 2013). Furthermore, the methods of measuring these parameters require specialised sample preparation and are not representative of entire consignments. These limiting factors have instigated the search for rapid and preferably non-destructive assessment techniques to predict maturity parameters of individual avocado fruit. Near infrared spectroscopy (NIRS) is an example of non-destructive technique that has been revealed as a precise, rapid and non-destructive alternative to destructive methods for providing non-visible information about comparative proportions of C-H, O-H and N-H bonds (Wetzel, 1983; Williams and Norris, 1987; Shenk et al., 2001; Workman and Shenk, 2004; Nicolaï et al., 2007; Magwaza et al., 2012).

2. Research hypothesis

This study attempts to evaluate the parameters to measure maturity of avocado fruit. The hypothesis is that TSS can provide a faster, easier, less expensive and reliable measurement of maturity in avocado fruit than the industry used oil content or MC. Furthermore, it is hypothesised that NIRS is a potential tool for non-destructive prediction of oil content, DM and MC of an avocado fruit.

3. Research aim

- To find a reliable, easy, less expensive and effective parameter for determining avocado fruit maturity that suitably represents the status of an avocado fruit.

4. Research objectives

- To evaluate and investigate various maturity parameters (MC, DM and oil content) of ‘Fuerte’ and ‘Hass’ avocado fruit.
- To investigate the possible use of TSS as an alternative parameter for determining maturity of avocado fruit.
- To investigate the reliability of NIRS in predicting maturity parameters (MC, DM and oil content) of avocado fruit.

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CHAPTER 2

EVALUATION OF MATURITY PARAMETERS OF AVOCADO FRUIT – A REVIEW

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Abstract

Maturity is one of the key factors that determine the quality of avocado fruit. The fruit does not exhibit physical characteristics that easily indicate its maturity status, hence, it is difficult for growers to judge whether or not the fruit is commercially ready for harvest. Current standards of determining maturity of avocado fruit are destructive and are with possible inaccuracies. This chapter review various indices of avocado fruit maturity and the possibilities of adopting non-destructive methods such as near-infrared (NIR) spectroscopy for predicting the maturity of the fruit. This chapter also explored the prospect of NIR-based imaging methods such as hyperspectral and tomographic imaging system and as prospective non-destructive techniques for avocado fruit maturity assessment.

Keywords: Avocado fruit, destructive methods, hyperspectral imaging, near-infrared spectroscopy, non-destructive assessment, tomography imaging.

1. Introduction

The avocado, *Persea americana* Miller, is a subtropical climacteric fruit originating from an area encompassing southern Mexico, Central America and the West Indies (Shepherd and Bender, 2002). The fruit is most commonly oblong with green exocarp (skin), although ‘Hass’, the dominant commercial cultivar, may become purple-black on the tree as the fruit approach maturity (Crane et al., 2013). Taxonomically, the fruit is divided into three horticultural races; namely the Mexican, the Guatemalan and the West Indian race (Bergh and Ellstrand, 1986; Liu et al., 2010; Chanderbali et al., 2013). A large number of avocado cultivars that exists today resulted from the hybridization among these avocado races (Knight, 1980; Bergh and Ellstrand, 1986; Newett, 2002). Major cultivars of economic importance in South Africa arose from the hybridisation of Guatemalan and Mexican races; these include ‘Fuerte’, ‘Hass’, ‘Pinkerton’ and ‘Edranol’ (Bijzet and Sippel, 2011; Crane et al., 2013).

The name ‘Fuerte’ meaning ‘strong’ in Spanish was given to this cultivar after it survived the extreme frost of January 1913 in California (Bijzet and Sippel, 2011). The shape of ‘Fuerte’ fruit varies from elongated with a long, narrow neck to dumpy with short, broad neck and can differ from medium to large size weighing between 170-500 g (Fig. 2.1A) (Crane et al., 2013). ‘Fuerte’ exocarp is green, thin, moderately glossy and easy to peel. The mesocarp has 18% oil content (Crane et al., 2013).

Globally, the green-skinned ‘Fuerte’ used to be the preferred cultivar of avocado fruit until the 1960s when it was replaced by ‘Hass’ (Crane et al., 2013). The replacement of ‘Fuerte’ by ‘Hass’ was as a result of inconsistent yield of ‘Fuerte’, but the fruit still retain its reputation in low humidity environments where diseases and insect pressure is low (Crane et al., 2013). ‘Fuerte’ still contributes significantly to the South African avocado industry as it comes onto the market two to four weeks earlier than ‘Hass’, enabling exporters to lengthen the export season and marketing time of South African avocados (Donkin, 2007; Lutge, 2011). Similarly, the cultivar still plays an important role in the French market that prefer ‘green skin’ avocado to the ‘black skin’ cultivars.

Fig. 2.1: A typical ‘Fuerte’ (A) and ‘Hass’ (B) avocado fruit.

‘Hass’ (Fig. 2.1B) is currently the leading avocado cultivar in terms of production (Crane et al., 2013), contributing about 90% to the global avocado trade (Crane et al., 2013). In the last

50 years, ‘Hass’ has become the dominant cultivar in subtropical climates and in 2010 accounted for 96% of production in New Zealand, 85% in California, 90% in Mexico, 57% in Peru, 75% in Chile, 75% in Spain, 80% in Australia, 58% in South Africa, 26% in Colombia and 35% in Israel (Crane et al., 2013). The cultivar is known for its superior pulp quality, higher yield and later maturing than ‘Fuerte’ (Crane et al., 2013). These attributes, including the fact that the exocarp turns purplish-black as it ripens, making colour change an indicator of ripeness (‘ready to eat’), contributed to ‘Hass’ popularity in the European market (Bijzet and Sippel, 2011). The flesh is of excellent quality with a nutty and rich flavour, mainly due to its high oil content of between 18 to 20% when matured (Crane et al., 2013).

2. Fruit growth and development

Fruit growth and development has received significant research interest (Schroeder, 1953, 1958; Robertson, 1971; McOnie and Wolstenholme, 1982; Van Den Dool and Wolstenholme, 1983; Zilkah and Klein, 1987a; Bower and Cutting, 1988; Sippel et al., 1992; Pak, 2002; Lakso and Goffinet, 2003). Monitoring fruit during growth and development is important in understanding the horticultural characteristics associated with fruit maturity stages. It is also helpful in identifying the optimum harvest time for a horticultural crop. Different horticultural crops exhibit different pattern of fruit growth and avocado fruit is arguably suggested to exhibit single-sigmoidal growth pattern as shown in Fig. 2 (Schroeder, 1958). Fruit growth, an irreversible increase in size, occurs basically via cell division and cell expansion or enlargement (Coombe, 1976; Lakso and Goffinet, 2003). Cell division is the first process that occurs in fruit after pollination and takes place simultaneously in many cells, thereby multiplying the number of cells in the fruit (Janick, 1986). Following cell division, the fruit begins a second phase of growth which is mainly accompanied by the enlargement of cells in the longitudinal, radial and tangential planes of fruit (Wareing and Phillips, 1970). However, a different pattern of growth has been observed in avocado fruit. Cummings and Schroeder (1942) and Barmore (1977) documented that a very rapid cell division takes place at the initial stage of avocado fruit growth. This stage is followed by cell enlargement that can stop at about 50% of its final size at maturity, and from thereon fruit size continues to increase via cell division.

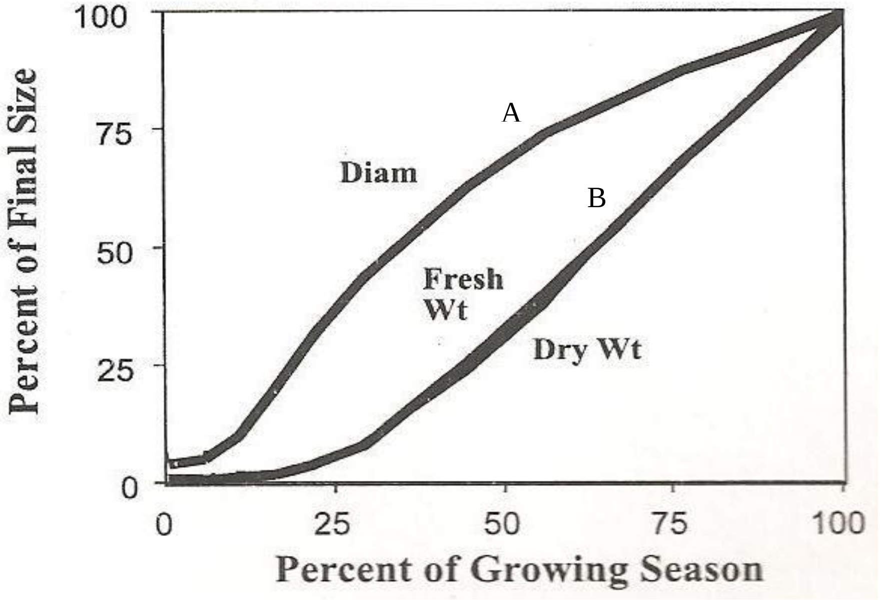

Fig. 2.2: Patterns of horticultural fruit growth (apple, pome fruit); single sigmoidal (A) and expolinear growth pattern (B).

Source: Lakso and Goffinet (2003)

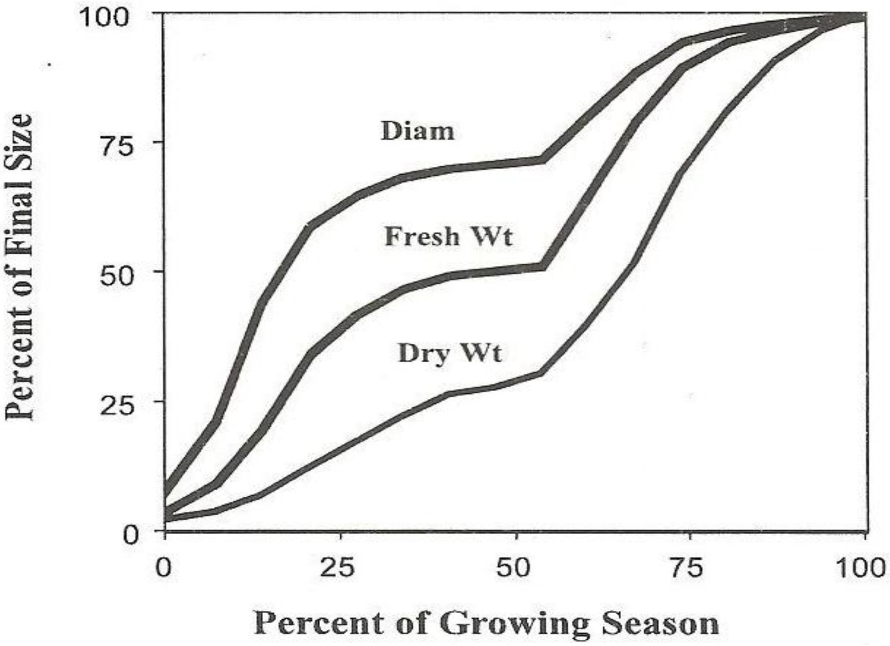

Fig. 2.3: Double sigmoidal growth pattern of horticultural fruit (stone fruit).

Source: Lakso and Goffinet (2003)

Fruit growth can either follow a single sigmoidal or an expolinear growth pattern, depending on the measured parameter (Fig 2.2) while other fruit display a double sigmoidal growth pattern (Fig. 2.3). Growth as observed in fruit does not occur at constant rate but undergoes certain patterns under normal and favourable conditions (Lakso and Goffinet, 2003). Overall, Lakso and Goffinet (2003) reported three main characteristic growth pattern of different fruit; these are single sigmoid, expolinear and double-sigmoid growth patterns. Most fruit exhibit the single sigmoid growth pattern which is characterized by an initial slow increase in size after bloom followed by a relatively rapid increase in size and then a slowdown in growth as fruit maturity is approached (Lakso and Goffinet, 2003).

In a fruit that exhibits an expolinear growth pattern, an early exponential curvilinear phase occurs during cell division similar to the sigmoid pattern but a linear growth is displayed during the cell expansion phase for the rest of the season (Lakso and Goffinet, 2003). The third category is fruit growth pattern of double-sigmoid form, which is typically observed in stone fruit. This growth pattern is characterized by initial exponential growth, during early cell division, followed by a significantly slower growth during the midseason to complete the first sigmoid phase. After this, a second rapidly growing phase occurs and then followed by a final slow growth as physiological maturity is approached (Lakso and Goffinet, 2003).

It has been reported that fruit growth and development in avocado fruit follow a single sigmoid growth pattern (Schroeder, 1953; Valmayor, 1964) with the exact pattern dependent on both, cultivar and method of measurement (Piper and Gardner, 1943; Blumenfeld and Gazit, 1974; Mougheith et al., 1978). The initial period of avocado fruit growth, irrespective of early or late maturing cultivar, is characterized by rapid cell division and cell enlargement in the mesocarp (Schroeder, 1953; Barmore, 1977). Although cell division in avocado fruit continues throughout its entire growth period, it occurs at slower rate during the later stages of development (Schroeder, 1953; Zilkah and Klein, 1987b; Cowan, 1997; Cowan et al., 1997). This means that growth never ceases as long as the avocado fruit remain attached to the tree (Schroeder, 1953; Valmayor, 1967; Van Den Dool and Wolstenholme, 1983). This continuous cell division even in a mature avocado fruit makes the fruit an uncommon species. The eventual avocado fruit size is not only determined by the initial rapid cell division and final cell size in the later period of growth, but also by cell multiplication and enlargement throughout the entire period of growth (Schroeder, 1953; Cowan, 1997; Cowan et al., 1997). However, Cummings and Schroeder (1942) documented that cell enlargement seized at about 50% size of the fruit at full maturity and that cell division was responsible for the continued growth of the avocado fruit. Factors like cultural practices, water relations and climatic conditions, are responsible for the variable cell sizes of in avocado fruit (Barmore, 1977; McOnie and Wolstenholme, 1982).

The most common maturity attributes of avocado fruit development is its concomitant decrease in mesocarp moisture content (MC) with a corresponding increase in the reciprocal value, dry matter (DM) (Pearson, 1975; Lee et al., 1983; Brown, 1984; Ranney, 1991). Similarly, several authors reported the accumulation of total lipid (fats and oil) during the growth and development of avocado fruit (Stahl, 1933a, b; Appleman and Noda, 1941; Lee et al., 1983; Brown, 1984; Ranney, 1991; Ranney et al., 1992). Hence, the most notable internal quality parameter that increases during fruit growth period is the oil content and it has relationship with the palatability (organoleptic) of the avocado fruit.

2.1. Fruit maturity

Fruit maturity involves the comprehension of developmental stages of fruit growth from flowering to maturity and, finally, senescence. The phases of fruit development can generally be divided into fruit growth, fruit maturation, attainment of physiological maturity, ripening and senescence (Watada et al., 1984; Kader et al., 1985; Watkins, 2003). Watada et al. (1984) defined growth as a permanent increase in observable features of a developing fruit, while maturation is the phase of growth and development preceding the completion of physiological or horticultural (commercial) maturity. Wills et al. (2007a) defined maturity as the fundamental component of fruit quality, particularly horticultural maturity. The authors defined physiological maturity as the phase of development of a fruit when ultimate growth has been achieved and the fruit has matured to such an extent that the following phase of development can be accomplished. Commercial or horticultural maturity, however, is concerned with the time of harvest as related to a particular end-use that can be translated into market requirement which often bears little relation to physiological maturity (Wills et al., 2007a). It is, therefore, important to allow horticultural products such as avocado fruit to reach their optimum maturity before harvesting and delivering to the market.

2.1.1. Fruit maturity indices/parameters

Having defined maturity, it is important to identify the actual stage during fruit growth and development when harvesting would be required so that growers can maximize their profits and consumers happy with their purchased products. To identify such stage, certain indications referred to as fruit maturity indices or parameters should be monitored in the fruit. Fruit maturity indices, which differ amongst different fruit, are measurements that can provide (an) indication(s) of fruit maturity (harvest maturity) (Reid, 1985). These indices, important factors during the postharvest marketing chain, are crucial to the eventual quality of fruit (Thompson, 1996). Examples of fruit maturity indices for various horticultural crops include skin colour, fruit size and shape, duration of development, firmness, total soluble solids (TSS), oil content, DM, MC and starch content (Thompson, 1996; Reid, 2002).

2.2. Avocado fruit maturity

Young and Lee (1978) defined maturity of an avocado fruit as the phase of development at which the fruit will ripen after harvesting resulting in an edible product. Similarly, Lee et al. (1983) defined physiological maturity of avocado as the phase of development where most internal components has reached their ultimate growth and horticultural maturity as the developmental phase where harvested fruit will undergo normal ripening to provide good eating quality. Parameters such as flavour, aroma, colour and texture are not obvious in avocado fruit until ripening is complete (Young and Lee, 1978); hence, judging the time of maturity of the fruit based on these parameters is difficult. It is noteworthy that avocado fruit can remain on the tree for up to 6 months or more even after reaching the stage of horticultural maturity (Schroeder, 1953; Hofman et al., 2013; Whiley et al., 2013). This distinct feature allows growers to use ‘late hanging’ of fruit on tree as a method of storing excess fruit for the late season market (Hofman et al., 2013); however, it is commercially essential to recognize the minimum maturity level that guarantees a satisfying eating experience and minimal fruit disorders which will allow early harvest to take advantage of higher early season prices (Hofman et al., 2013). Recognizing minimum harvest maturity and harvesting fruit as early as possible could also reduce the risk of fruit loss from theft or weather damage (wind, hail or frost), or from fungal diseases (Robert Blakey, pers. com. 2014). The need for minimum maturity standard have led to the discovery of some maturity parameters, such as oil content (Lee et al., 1983; Kaiser and Wolstenholme, 1994), DM (Lee et al., 1983; Brown, 1984; Ranney, 1991) or MC (Pearson, 1975), changes in fruit growth rate (Plumbley et al., 1989) and fruit size (Sippel et al., 1992; Hofman and Jobin-Decor, 1999). Various avocado industries determine these parameters using destructive methods which have various limitations. These limitations include expensive and time consuming nature of measurement (associated with the purchase of chemicals and laboratory equipment) as well as difficulty of measuring the oil content (Hofman et al., 2013). Other shortcomings include variability among cultivars and between production locations (Hofman et al., 2013). These limitations call for studies to develop rapid, easy, reliable, and less expensive techniques for determining avocado fruit maturity. Non-destructive methods would be preferable as they are capable of providing rapid analysis of individual fruit.

2.2.1. Current parameters for determining avocado fruit maturity

Fruit maturity requires determination of some characteristics known to change as fruit develop (Wills et al., 2007b). In avocado, current maturity parameters include oil content, MC (used as the maturity parameter in South Africa avocado industry) and the complementary value to MC, DM (used in avocado fruit producing countries other than South Africa) (Pearson, 1975; Swarts, 1978; Lee et al., 1983; Kaiser and Wolstenholme, 1994; Hofman et al., 2002).

2.2.1.1. Oil content

The oil content of avocado fruit increases after fruit set; this increase has been reported to be directly proportional to the age of the fruit (Ozdemir and Topuz, 2004). The maturity parameter increases during the entire growth and development of the fruit (Ozdemir and Topuz, 2004; Hofman et al., 2013) with likely alterations caused by climactic factors, such as temperature and rainfall (Kruger et al., 1999). Such alteration was observed as oil content accumulation increased due to heavy rainfall as against the general believe that oil content increases at faster rates during warmer months (Kruger et al., 1999). The concentration of oil in the pulp of avocado fruit is an important factor determining the eating quality of fruit (Hofman et al., 2013). Therefore, oil content has been reported as a dependable maturity parameter of avocado fruit (Lee et al., 1983; Kaiser, 1994; Kaiser and Wolstenholme, 1994; Hofman et al., 2002). Due to the difficulty and expensive nature of determining oil content, alternative methods of measuring avocado fruit maturity are currently researched (Hofman et al., 2013).

The use of near-infrared (NIR) spectroscopy (NIRS) has been suggested as an alternative method of determining maturity of avocado fruit as the technique is capable of providing rapid, non-invasive measurement of maturity parameters. This would be possible since the NIR spectra could be modelled against the wet chemistry data of the fruit (Schmilovitch et al., 2001; Whiley et al., 2002; Blakey, 2011; Wedding et al., 2011). The NIRS is a method that uses the near-infrared region of the electromagnetic spectrum between 780 and 2500 nm (Nicolaï et al., 2007) where a given sample is subjected to NIR radiation and the reflected or transmitted radiations are captured as spectra. The captured spectra are thereafter subjected to multivariate statistical analysis to derive the necessary relationship with the parameters of interest (Nicolaï et al., 2007).

2.2.1.1.1. Destructive methods of determining oil content

The concentration of avocado oil can be determined by Soxhlet extraction using petroleum ether according to the International Union of Pure and Applied Chemistry (IUPAC) method 1.122 (IUPAC, 1979). This method, however, has certain disadvantages as recognized by Lee et al. (1983) in that it is extremely time consuming. Similarly, a relatively faster refractometric method of determining the oil content of avocado fruit was suggested by Lesley and Christie (1929). This method involves the use of Halowax oil (monochloronaphthalene) as a solvent and a refractometer (Lee, 1981a). The limitations of the method includes the inconsistency of refractive index of the Halowax oil, temperature-dependent readings, many procedural transfers, expensive equipment and the suspected carcinogenic nature of the Halowax oil that had to be taken off the market (Lee et al., 1983). These shortcomings made the method inappropriate for the majority of growers of avocado fruit (Lee, 1981b). Meyer and Terry (2008) described a method to quantify the oil content of avocado fruit from freeze-dried mesocarp tissue samples using Hexane. The advantage of this method is that other chemical components such as sugars can be quantified using the residue from the same sample after the oil content has been determined.

2.2.1.2. Mesocarp moisture content

As a result of the unavailability of a quick, easy and inexpensive method of measuring the oil content, the MC and its reciprocal, the DM, has been used as maturity indicator by avocado industries in numerous countries (Blakey, 2011). South African avocado industry prefers the use of MC as avocado maturity index while the avocado industries of Australia, Israel, New Zealand, Chile and the United States use the DM. Hence, DM of avocado fruit flesh has been evaluated as a maturity index by several authors and has been found to be associated with oil content and avocado fruit maturity (Lee et al., 1983; Brown, 1984; Ranney, 1991). Nevertheless, Blakey (2011) documented that MC of avocado fruit varies with possibilities of recording low MC regardless of the fruit maturity. This low MC could either be induced by water-stress conditions or fruit exposure to direct sunlight while attached to the tree. Similarly, Sippel et al. (1992) and Hofman and Jobin-Décor (1997) suggested that smaller fruit could have a higher DM compared to the larger fruit; this could be due to the earlier maturity caused by premature seed coat senescence (Hofman et al., 2013). In view of this, MC may not be a reliable indicator of avocado maturity.

2.2.1.2.1. Destructive methods of determining mesocarp moisture content of avocado fruit

The MC of avocado fruit can be estimated by determining fresh and dry mass of a core sample fruit (Sugiyama and Tsuta, 2010). The exocarp, the seed and the seed coat of the cored portion should be removed and discarded before recording fresh mass. The free water of the cored portion of the sample fruit can either be removed by drying the fruit sample using an oven or a freeze dryer (Sugiyama and Tsuta, 2010). The MC, or its reciprocal value, DM, can be calculated using the following equations 1 or 2, respectively;

$$\mathrm{MC} = \frac{\mathrm{M_f - M_d}}{\mathrm{M_f}} \times 100 \qquad (1)$$

Where:

MC represents mesocarp moisture content

M_f represents fresh mass of the core sample

M_d represents dry mass of the core sample.

$$\mathrm{DM} = \frac{\mathrm{M_f - M_d}}{\mathrm{M_d}} \times 100 \qquad (2)$$

Where:

DM represents the dry matter

M_f represents fresh mass of the cored sample

M_d represents dry mass of the cored sample.

3. Non-destructive technologies for evaluating avocado fruit maturity

Advancement in science and technology has launched several non-destructive methods of analysing biological and chemical components of agricultural products (Zerbini, 2006). Kim et al. (1999) pointed out the increasing importance of determining the quality of agricultural products non-destructively, so as to be able to grade such produce on-line. Upgrading the value of these products through quality sorting has amplified investigations into related techniques for determining the quality of products. Techniques such as physical, acoustical, electrical, and optical analysis as well as X-ray and nuclear magnetic resonance (NMR) have been found suitable for determining specific quality attributes of agricultural products (Kim et al., 1999).

In avocado fruit, several technologies can be used to determine the maturity status of the fruit non-destructively. Some of these technologies include magnetic resonance imaging (MRI) and NIRS. The former is an application of NMR and is an analytical tool used for chemical analysis. Application of NMR as well as MRI have successfully provided fast and non-destructive means of analysing matter from the atomic to the macroscopic scale (ACS, 2011). Several authors have recorded successes in using MRI to determine DM and thereby avocado fruit maturity (Chen et al., 1993). Similarly, the technique has been successfully applied to other horticultural fruit (Wang and Wang, 1989; Gonzalez et al., 2001; Sánchez et al., 2003). However, small sample sizes, slow speed of processing data and high cost of the equipment are some factors that limit the potential use of MRI to determine avocado fruit maturity on an industrial scale (Clark et al., 1997; Clark et al., 2003c).

The maturity of a consignment of avocado fruit is currently monitored destructively using only a few representative samples (Blakey, 2011). However, the importance of measuring the maturity of all fruit in a consignment by a non-destructive method has been noted by Hofman et al. (2002). According to Schmilovitch et al. (2001) and Clark et al. (2003a), the use of NIRS in determining maturity parameters of avocado fruit is minimal in spite of its promising potentials. Nonetheless, Wedding et al. (2013) reported promising results using NIRS to predict the DM of ‘Hass’ avocado fruit. This means that measuring avocado fruit non-destructively could be achieved by avocado industries, particularly, as NIRS provides a fast approach to perform analysis, as it takes between 0.1 to 10 seconds for full range scanning to measure compounds with concentrations that is as low as 0.1% (Burns, 2001). Using NIR technology to measure moving fruit on a packing line with 8-10 fruit per seconds could possibly threaten its compatibility but with the advent of newer technologies, it is feasible to apply the technology in pack houses.

4. Potential use of NIRS to predict maturity parameters of avocado fruit

The need for measuring the maturity of individual avocado fruit and their internal quality non-destructively has spurred investigations into the potential use of NIRS as a means of non-destructive fruit maturity measurement (Hofman et al., 2002). An extensive literature review on NIRS, covering its origin, principles, modes of spectra acquisition, method of use and chemometrics has been carried out by Nicolaï et al. (2007) and Magwaza et al. (2012).

The ability of avocado growers to consistently deliver an avocado fruit of excellent qualities to consumers will explicitly improve profitability and competitiveness of the avocado industries (Wedding et al., 2010). High prices associated with early season avocado fruit in the market make some growers harvest immature fruit for the benefit of more financial gains. Shipping avocado fruit earlier to supply the European market at a slightly earlier time to obtain better prices can result in harvesting immature avocado fruit. Immature avocado fruit can display poor fruit quality when fully 'eat-ripe', thereby causing unpalatable fruit and hence consumer dissatisfaction (Schmilovitch et al., 2001). In view of this, recent research has focused on using NIRS to predict avocado fruit maturity parameters (Clark et al., 2003c; Wedding et al., 2010). Kawano (2002) and Walsh and Subedi (2010) argued that predicting DM and oil content of avocado fruit mesocarp is possible with the use of NIR technology. Such technology can be used to quantify the oil, sugar and protein concentration of a given commodity, thereby increasing profitability as a result of production expansion (Walsh and Subedi, 2010). Many reports have focused on the accuracy of using NIRS to predict the DM and ultimately determine avocado fruit maturity (Clark et al., 2003c; Wedding et al., 2010; Wedding et al., 2011). Schmilovitch et al. (2001) developed an NIR calibration model for maturity (R^2 = 0.90) with a standard error of prediction of 1.3%. A goodness-of-fit (R^2) between predicted and actual DM measurements of 0.88 with an error of prediction of 1.8% for DM was modeled by Clark et al. (2003b). Wedding et al. (2010) tested the use of NIRS in estimating the DM of 'Hass' avocado fruit and obtained a good prediction statistics of (R^2 = 0.76; root mean squared error of prediction (RMSEP) = 1.53%). Furthermore, Wedding et al. (2013) recognised the importance of robustness in calibration model development for DM in avocado fruit as the predictive value becomes more accurate (R^2= 0.89 and RMSEP = 1.43%) for DM when more samples from the later seasons were incorporated in the model.

5. Prospects for future research

Non-destructive maturity evaluation of avocado fruit is gradually becoming successful as researchers are continuously exploring appropriate technologies viable for such purposes. One of the most creative non-destructive technologies developed to evaluate both internal and external qualities of horticultural products is the NIR technology (Magwaza et al., 2012). Research activities have examined a number of NIR applications in determining various maturity parameters of avocado fruit; however, researchers should also consider other applications of NIR such as hyperspectral and tomographic imaging systems for maturity and quality evaluation of avocado fruit.

Hyperspectral imaging (HSI) is a technique that produces spatial map of spectral variation of certain tissues (ElMasry and Sun, 2010). The ability of the technique to automatically identify an object and provide details of its analytical composition makes it an interesting area of research that could be of benefit to avocado fruit industries. The technique integrates spectroscopy and conventional imaging systems to provide spatial distribution and spectral information of a sample (Chen et al., 2002; ElMasry et al., 2007; Gowen et al., 2007). Apart from its non-destructive nature, other advantages of HSI include minimal sample preparation, sensitivity to minor components, fast acquisition times, and simultaneously visualizing the spatial distribution of several chemical compositions (Gowen et al., 2007; ElMasry and Sun, 2010). This technique has been successfully implemented in a wide range of horticultural products such as strawberry (ElMasry et al., 2007), litchi (Liu et al., 2014), apple (Kim et al., 2002), kiwifruit (Martinsen and Schaare, 1998), banana (Rajkumar et al., 2012), melon (Sugiyama and Tsuta, 2010), tomato (Polder and van der Heijden, 2010), mushroom (Gowen, A. A., 2010) cucumber (Ariana et al., 2006) and citrus (Ye et al., 2006; Gómez-Sanchis et al., 2008; Okamoto and Lee, 2009; Qin et al., 2009; Molto et al., 2010; Lorente et al., 2012; Qin et al., 2013); hence, successful implementation of HSI in avocado fruit industry could be possible.

Optical tomography (OT) is a further non-destructive analytical technique appropriate for the investigation of internal structure of biological tissues (Magwaza et al., 2012). The technique recreates the 3D images of a targeted object through transmission and scattering of light. Diffraction tomography, diffuse optical tomography and optical coherence tomography (OCT) are the three existing central approaches to OT. However, OCT provides a more suitable approach for the evaluation of internal structures of biological tissues than other approaches (Huang et al., 1991; Fercher et al., 2003; Lu et al., 2004). Thus, OCT is a novel imaging technology, capable of providing visual analyses of internal microstructure of biochemical components of agricultural products (Fujimoto, 2003; Meglinski et al., 2010; Magwaza et al., 2012). The technique is capable of providing non-destructive high-resolution (1-15µm) cross-sectional tomographic imaging of objects (to the depth of 1-1.5mm) in a real time mode (Meglinski et al., 2010; Magwaza et al., 2012). Previously, OCT has only been applied to the biomedical industries for the monitoring of blood sugars in diabetic patients, diagnosis of tumours (Sapozhnikova et al., 2003; Zagaynova et al., 2005; Sapozhnikova et al., 2006). It has also been used to visualize lipid distribution within arterial vessel walls (Hirano et al., 2014).

Similarly, it is currently explored for image analyses of internal and external qualities of horticultural products such as apple (Verboven et al., 2013) and onion (Ford et al., 2011; Landahl et al., 2012). The results of these studies have been promising and probable use of this technique in providing visual analysis of maturity parameters of avocado fruit is suggested to be researched.

6. Conclusion

Harvesting avocado fruit at full maturity directly affect the eventual eating quality of the fruit and allows producers to take advantage of the early market. However, growers tend to harvest fruit before the commodity reaches a palatability that satisfies the consumer. Based on estimate, destructive methods are used to determine compounds aligned with maturity of all fruit in a consignment but a non-destructive technique will provide the actual maturity status of an individual fruit with respect to a specific parameter. Therefore, individual fruit in a consignment should be analysed and confirmed to have attained the acceptable minimum maturity prior to market delivery. This will allow farmers to maximise profits and yet satisfy consumers by delivering avocado fruit of good quality to the market. To analyse individual fruit for maturity, non-destructive methods of evaluating the fruit maturity parameters should be employed. The NIRS, regarded as the most advanced among the available non-destructive techniques, offers prospects for the measurement of the internal chemical compositions of avocado fruit. Although evaluation of DM of various fruit commodities is possible, no literature has reported the application of the NIRS technology to evaluate the internal chemical compositions of avocado fruit, such as oil content and TSS concentration. Applications of NIR, such as HSI and OCT are other creative, reliable, and cost-effective technologies that offer prospect to the avocado fruit industry for non-destructive evaluation of internal maturity parameters.

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CHAPTER 3

GROWTH AND DEVELOPMENTAL PATTERN OF ‘FUERTE’ AND ‘HASS’ AVOCADO FRUIT FROM THE COOL SUBTROPICAL AREA OF KWAZULU-NATAL

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Abstract

The growth pattern of avocado fruit is said to follow a single sigmoidal growth curve. This pattern is commonly determined by measuring fruit length and/or diameter. However, the increase in avocado fruit diameter has been reported to be perpetual and more stable than fruit length. This stable increase in size of fruit diameter suggests that diameter is probably a better indicator of avocado fruit growth pattern than length. Fruit diameter and fruit mass were measured bi-weekly from February to March and then monthly from April to August from two commercial orchards, Everdon Estate and Bounty Farm, both located in the cool subtropical area of KwaZulu-Natal, South Africa. Fruit growth results indicated that avocado fruit from Everdon Estate grew faster in diameter and matured faster than those from Bounty Farm. Results of growth pattern revealed that avocado fruit exhibits a single sigmoidal growth pattern but one of the cultivars exhibited a tendency for double sigmoidal growth pattern.

Keywords: Avocado, double sigmoid growth curve, single sigmoid growth curve.

1. Introduction

The growth and development of horticultural crops is an important area of research, as it assists in determining the optimum harvest maturity of fresh produce. In most fruit, after fruit setting, cell division which determines the size of harvested fruit is dominant while little cell expansion occurs. This is followed by the completion of the cell division phase (Luckwill, 1959; Ezura and Hiwasa-Tanase, 2010), and further growth occurs primarily by cell expansion (Lakso and Goffinet, 2003). More recently, the initial period of avocado fruit growth is characterized by rapid cell division which is then followed by cell expansion, has been reported to be hormonally regulated through isoprenoid metabolism (Cowan et al., 1997). Cell division and expansion are stimulated by hormones such as auxins (Nitsch, 1953), gibberellins and cytokinins (Crane, 1964).

Avocado fruit is reported to follow a single sigmoid pattern of growth (Schroeder, 1953). This pattern is commonly determined by measuring fruit length and/or diameter (Dixon et al., 2006). However, the increase in avocado fruit diameter has been reported to be perpetual and more stable than fruit length (Van Leylyveld, 1978; Cowan et al., 1997). This stable increase in size of fruit diameter suggests that diameter is probably a better indicator of avocado fruit growth pattern than length.

Other fruit types such as apple, pear, pineapple, banana, strawberry, orange, tomato and melon are examples of fruit that exhibit a single sigmoidal growth pattern, while stone fruit and nuts, grapes, figs and olives are known to exhibit a double sigmoidal growth pattern (Crane, 1964;

Coombe, 1976; Ezura and Hiwasa-Tanase, 2010). Fruit that exhibit single sigmoidal growth pattern are characterised by expanding slowly during the initial and eventual period of growth as well as at a considerable faster rate during the middle developmental phase (Lakso and Goffinet, 2003; Ezura and Hiwasa-Tanase, 2010). Most authors, however, explained that fruit exhibiting a double sigmoidal growth pattern have two phases of rapid growth separated by a phase of relatively slow growth. The slow growth period also referred to as plateau phase in the double sigmoidal growth pattern, is commonly associated with the period of seed (Ezura and Hiwasa-Tanase, 2010) or stony endocarp hardening (Luckwill, 1959).

Avocado fruit is believed to exhibit single sigmoidal growth patterns but because of its hard seed, the fruit is suggested to be an atypical fruit that exhibit a single sigmoidal growth pattern. As a large seeded oil-accumulating fruit, it is hypothesized that avocado fruit may display the characteristics of a double sigmoidal growth pattern observed in most nut and stone fruit. Additionally, the fact that avocado fruit does not ripen on the tree (Bower and Cutting, 1988) could point towards a second growth phase that occurs after the fruit is commercially harvested. Therefore, this study was conducted to verify the type of growth pattern followed by avocado fruit.

2. Materials and methods

2.1. Fruit material

During the 2014 growing season, 'Fuerte' and ‘Hass’ avocado fruit were harvested from Bounty Farm (Latitude: 29°28´S; Longitude: 30°161´E) and Everdon Estate (Latitude: 29°45’S, Longitude: 30°25’E), both located in the cool subtropical area of KwaZulu-Natal, South Africa. Fruit sampling started from February (golf ball size) to August (after commercial maturity). During each sampling date, 5 ‘Fuerte’ and 5 ‘Hass’ avocado fruit were measured on 5 trees for each of the cultivars at the two farms. Moreover, 40 ‘Fuerte’ and 40 ‘Hass’ avocado fruit were harvested at random from 20 mature trees per cultivar at each location for fruit mass measurement. Although both locations are cool subtropical areas, Everdon Estate was characteristically slightly cooler than Bounty Farm and trees were under irrigation (Table 3.1). Bounty Farm is solely dependent on rainfall. Harvested fruit were put in crates and immediately transported in a well-ventilated vehicle to the postharvest laboratory in the University of KwaZulu-Natal where the fruit mass was measured.

2.2 Measurement of fruit mass

Individual fruit harvested was measured and recorded using a weighing balance. Measurements were taken on a bi-weekly basis from 14/02/2014 to 20/03/2014 and later on monthly basis from 20/03/2014 to 03/09/2014.

2.3 Measurement of fruit growth

Five individual fruit were randomly marked on each of the five trees used for this experiment. Individual fruit diameter was measured using digital callipers while fruit still attached to the tree. Measurements were taken on a bi-weekly basis from 14/02/2014 to 20/03/2014 and later on monthly basis from 20/03/2014 to 03/09/2014.

2.4 Statistical analysis

Experiments were laid out using completely randomized design (CRD) using individual fruit as a replicate. Data for the analytical determinations were subjected to analysis of variance using GenStat® 16th Edition (VSN International, Hemel Hempstead, UK) and the means were separated by Duncan's multiple range test. Differences at $P \leq 0.05$ were considered to be significant.

3. Results

There were significant differences between production locations and day of measurements in both, 'Fuerte' and 'Hass' avocado fruit (Figs. 4.1, 4.2, 4.3, 4.4 and 4.5). The trends in diameter (Fig. 3.1A and Fig. 3.2A) were fairly consistent over the measuring periods. There were significant differences ($p < 0.001$) in terms of diameter between different stages of development and between production location for both 'Fuerte' and 'Hass' avocados (Fig. 3.1A and Fig 3.2A). For both cultivars, a rapid increase in diameter was recorded at the beginning of the sampling period and then followed by a linear growth. 'Fuerte' fruit from Bounty Farm had a continuous growth trend exhibited by a significant increase in diameter as observed during the last stage of data collection.

3.1 Fruit growth

There were highly significant differences in fruit growth in terms of diameter between different stages of development and between production location for both 'Fuerte' and 'Hass' avocados ($p < 0.001$), but the interaction between harvest time and orchard location was not significant with p-values of 0.840 and 0.314, respectively. For both cultivars, a rapid increase in diameter was observed at the beginning of the sampling period, followed by a linear growth or a 'plateau' as a result of cooler and drier weather experienced during winter months of the sampling period (Table 3.1). A shrinkage in size was noticed for 'Fuerte' fruit from Bounty Farm between day 94 (63.11 mm) and day 128 (62.33 mm) which would have been caused by the cool weather condition (Table 3.1). 'Fuerte' fruit from Bounty Farm had a continuous growth trend exhibited by a significant increase in diameter as observed during the last stage of data collection. Results presented in Fig. 3.1A and Fig. 3.1B showed that the diameter of fruit harvested from Everdon Estate increased faster than the ones from Bounty Farm. There was no evidence of a significant increase in fruit growth from July until the last day (247th day of the year) of data collection, except for 'Fuerte' fruit from Bounty Farm which significantly increased between 213th (August) and 247th (September) day of the year.

3.2 Fruit mass

Highly significant differences (< 0.001) in fruit mass of fruit harvested from Bounty Farm and Everdon Estate were recorded for both 'Fuerte' and 'Hass' fruit. Although significant interaction between harvest date and production location were observed, it is important to take note of the interaction between these factors as these indicate that the difference in fruit mass was highly dependent on both harvest time and orchard location.

4. Discussion

4.1 Fruit growth

Fruit growth can be determined by measuring volume, fresh mass, length, diameter of fruit or combination of these parameters. If the increase in size in terms of volume, fresh mass or diameter of a fruit is plotted against time after anthesis, the resulting pattern of growth may either be single sigmoidal or double sigmoidal in nature (Jackson, 2011). In this study, fruit fresh mass and the diameter of avocado fruit were measured and an overall increase in fruit mass and diameter were recorded throughout the entire period of study.

For ‘Fuerte’ fruit, the mass of Bounty and Everdon Estate fruit ranged from 109.3 to 259.6 g and 124.3 to 261.7 g (Fig. 3.1B), while ‘Hass’ fruit ranged from 91.1 to 221.7 g and 121.6 to 246.4 g (Fig. 3.2B) respectively. The mass of ‘Fuerte’ fruit increased consistently, reaching a peak between 08/05/2014 and 03/06/2014 with a sudden decline on 04/07/2014 (Fig. 3.1B). The decline could be attributed to the unavailability of bigger fruit as the farms had begun their commercial harvesting. A similar trend was observed for ‘Hass’ fruit, as it also increased consistently over the entire period of study with a tendency to continue increasing in size (Fig. 3.2A). It could also be deduced that fruit from Everdon Estate grew faster and bigger than fruit from Bounty Farm owing to higher availability of water at Everdon Estate due to the present irrigation system. A linear fruit growth pattern in terms of fresh mass was observed for the two cultivars from both production locations, but this measurement could not be used to determine the actual pattern of avocado fruit growth. This is because different fruit were measured at every sampling date; hence, the actual increment in size of a single fruit could not be established for each fruit. However, some individual fruit were marked on the tree and measured for diameter throughout the entire period of the experiment (Fig. 3.1A and Fig. 3.2A). Therefore, diameter data can be used to determine the pattern of avocado fruit growth (Van Leylyveld, 1978).

The overall growth of both cultivars in terms of fruit diameter from both production locations exhibited a similar growth pattern (Fig. 3.1A and Fig. 3.2A). A rapid increase in size as evident in both, ‘Fuerte’ and ‘Hass’ avocado fruit, revealed the occurrence of a rapid growth rate between the 45th and 94th day of the year 2014 corresponding to between February and April (Fig. 3.1A). For ‘Fuerte’ and ‘Hass’ fruit from Bounty Farm and Everdon Estate, a reduction in growth rate occurred during the cool period of the year between 128th (May) and 185th (July) day of the year (Fig. 3.1A and Fig. 3.2A). This could be attributed to the cool dry winter weather (Table 3.1). The increase in size of both cultivars appeared to follow a single sigmoidal growth pattern as previously reported (Schroeder, 1958; Zilkah and Klein, 1987; Morre-Gordon et al., 1995; Liu et al., 1999; Pak, 2002). The significant increase in fruit growth recorded for ‘Hass’ fruit during the last day of data collection, however, suggests a resumption of fruit growth. This experimental result was similar with the results reported by McOnie and Wolstenholme (1982) and Pak (2002) which could in fact suggest that avocado fruit follows a double sigmoidal growth pattern. The linear growth phase as observed in this experiment shares close similarities with the morphological studies of fruit growth as reported in previous studies (Cummings and Schroeder, 1942).

Similar to the data recorded for fruit mass, fruit growth in terms of diameter (mm) revealed that fruit from Everdon Estate grow faster than those from Bounty Farm, even though the two farms are located in a cool subtropical area. The diameter of ‘Fuerte’ fruit from Bounty Farm and Everdon Estate ranged from 50.9 to 67.5 mm and 56.0 to 69.6 mm (Fig. 3.1A) while ‘Hass’ fruit ranged from 48.9 to 62.5 mm and 55.5 to 70.7 mm (Fig. 3.2A), respectively. As stated earlier, Bounty Farm, unlike Everdon Estate, relies on rainfall as the sole water source.

This lack of cultural practise could have contributed to the production of smaller sized fruit compared with the ones from Everdon Estate which are bigger size wise. This is similar with earlier reports on the effect of irrigation on the size of avocado fruit (Lahav and Kalmar, 1977; Bower, 1985; Arpaia and Eaks, 1990; Vuthapanich et al., 1998; Carr, 2013).

5. Conclusion

The growth of ‘Fuerte’ and ‘Hass’ avocado fruit from the cool subtropical area of KwaZulu-Natal appeared to follow a single sigmoidal growth pattern except for ‘Fuerte’ fruit from Everdon Farm which showed a tendency of re-growth suggesting a double sigmoidal growth curve. However, results reported in this study are based on one season data and are not conclusive. Therefore, an extended period of study is suggested.

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Table 3.1: Minimum and maximum temperatures in the experimental avocado production area (Howick)

Month	January	February	March	April	May	June	July	August	September	October	November	December
mm	133	128	114	51	24	15	16	21	37	83	109	130
ºC (min)	20.5	20.5	19.4	16.9	13.7	10.7	10.6	13.0	15.5	17.5	18.2	19.8
ºC (max)	26.4	26.4	25.3	23.7	21.3	19.0	18.9	21.2	22.9	24.1	24.5	25.7

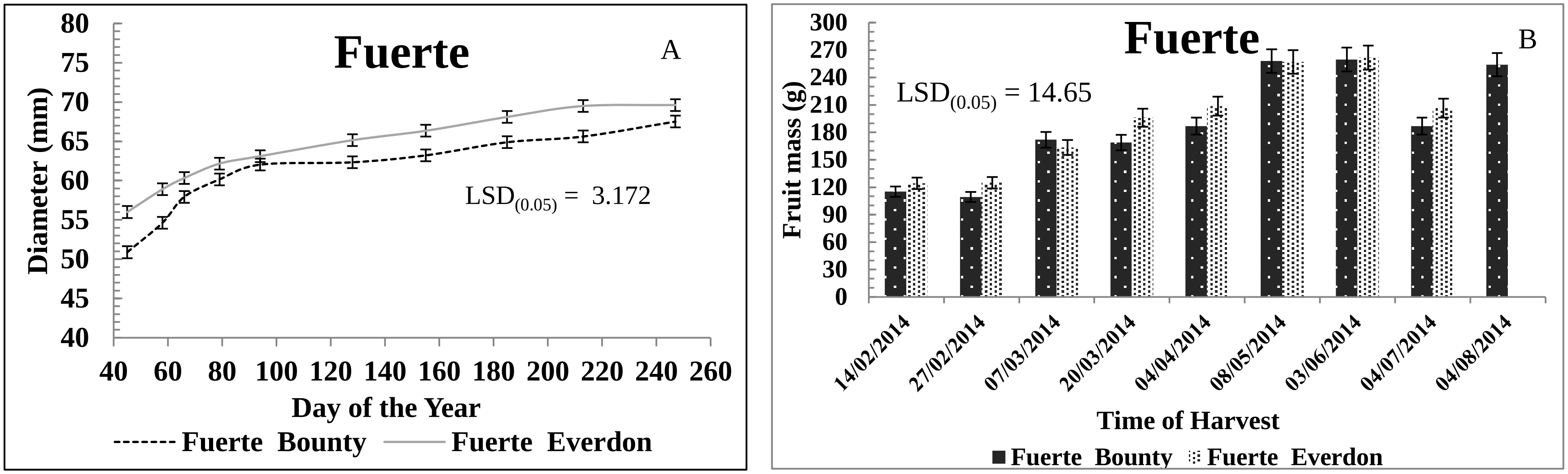

Fig. 3.1: Changes in diameter (A) and fruit mass (B) of 'Fuerte' avocado from two commercial production locations in the cool subtropical area of KwaZulu-Natal, South Africa during the 2014 growing season.

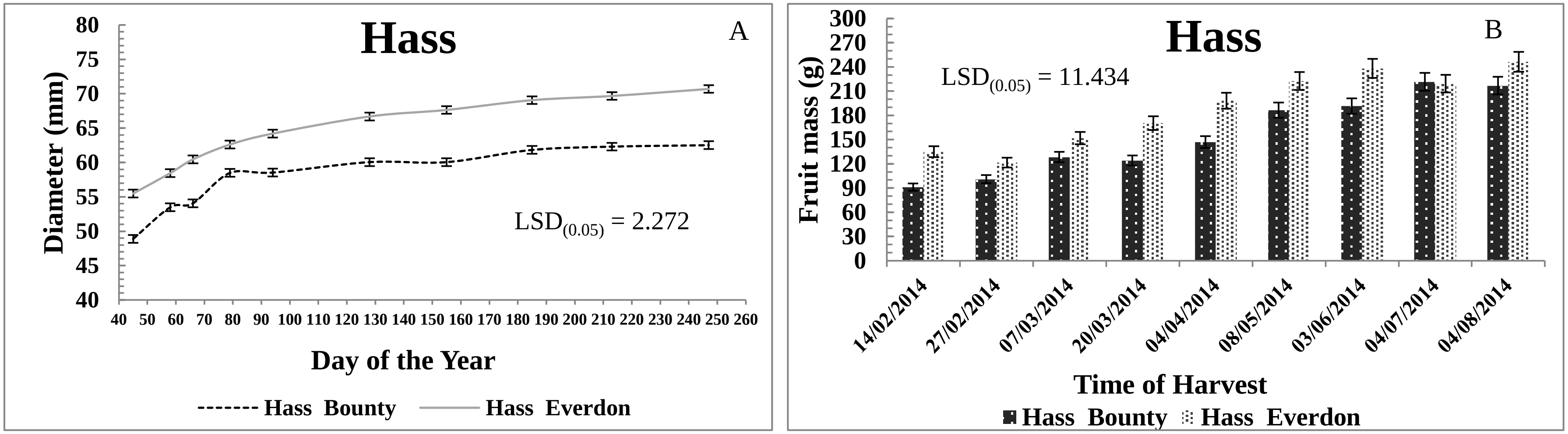

Fig. 3.2: Changes in diameter (A) and fruit mass (B) of 'Hass' avocado from two commercial production locations in the cool subtropical area of KwaZulu-Natal, South Africa during the 2014 growing season.

CHAPTER 4

EVALUATING TOTAL SOLUBLE SOLIDS AS AN ALTERNATIVE PARAMETER OF AVOCADO FRUIT MATURITY

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Abstract

The exact harvest time of avocado fruit is difficult to determine as the fruit lack obvious physical and visual indications that could easily depict maturity. It is therefore, important to harvest mature avocado fruit as only a palatable non-shrivelling fruit ensures higher consumer acceptability and good market value. Total soluble solids (TSS) have been employed as an indicator of harvest maturity for many horticultural crops but not commonly in oil-accumulating fruit. Avocado fruit maturity has, however, recently been related to its mesocarp C7 sugars content. During 2013 season, 'Fuerte’ and ‘Hass’ avocado fruit were sampled from Bounty Farm located in the cool subtropical area of KwaZulu-Natal, South Africa. Fruit were harvested during the early, mid and late harvesting period. In 2014, samples of the same cultivars were collected bi-weekly from the two farms, Bounty Farm and Everdon Estate, starting from February to August. TSS was measured on the equatorial region of each fruit using a digital refractometer. Different physico-chemical properties such as fruit mass, mesocarp moisture content (MC), dry matter (DM), and oil content were also evaluated during growth and development to understand growth pattern of avocado fruit. The main objective of this study was to investigate the use of TSS as an alternative maturity indicator to determine harvest maturity of ‘Fuerte’ and ‘Hass’avocado fruit. For both ‘Hass’ and ‘Fuerte’ and in both seasons, TSS was significantly different between sampling dates ($p < 0.001$) and was also significantly different between production locations during the growing period of 2014 season ($p < 0.001$). The MC decreased at a constant rate, from 87.3 to 69.4% and 86.8 to 66.3% for ‘Fuerte’ fruit as well as from 85.2 to 69.4% and 86.6 to 68.3% for ‘Hass’ fruit, both from Bounty Farm and Everdon Estate, respectively over the sampling period. Oil content on the other hand increased from 10.2 to 24.9% and 12.3 to 26.9% for ‘Fuerte’ avocado fruit as well as 7.5 to 30.5% and 13.0 to 27.7% for ‘Hass’ avocado fruit from Bounty Farm and Everdon Estate, respectively, although, the rate of increase was not constant. The results obtained for TSS during the growing season fluctuated and did not follow a particular pattern, indicating that TSS cannot be used as an indicator of avocado fruit maturity. This study further demonstrated that MC is a better indicator of maturity than other physico-chemical properties.

Keywords: Avocado, fruit maturity, mesocarp oil, moisture content, total soluble solids.

1. Introduction

Avocado (*Persea americana*, Miller) fruit is a unique climacteric fruit and, unlike other fruit, does not exhibit obvious physical changes during its growth and development that could be perfectly related to maturity (Lee, 1981). Hence, it is difficult to judge whether the fruit has matured sufficiently to be palatable. (Lee, 1981). Harvesting immature avocado fruit can have a negative effect on fruit quality resulting in grassy aftertaste, watery or rubbery texture, and lack of flavour (Harding, 1954; Hofman et al., 2013). Avocado fruit harvested immature will also shrivel, exhibit poor storage or eating quality, and take longer time to soften (Hofman and Jobin-Décor, 1997). Furthermore, harvesting immature avocado fruit could lead to commercial losses due to consumers' lack of interest in purchasing bad fruit. Therefore identifying the optimal harvest time is an important factor that determines the growers' profitability and consumer satisfaction.

Various parameters for determining maturity of avocado fruit have been suggested to include oil content, mesocarp moisture content (MC) and dry matter (DM) as parameters for determining maturity of avocado fruit (Ozdemir and Topuz, 2004; Dodd et al., 2008; Gamble et al., 2010; Hofman et al., 2013). Hodgkin (1939) was the first to document a relationship between the oil content and avocado fruit quality. However, the variability in oil content within fruit from the same tree has questioned this parameter as a maturity measure of avocado fruit (Obenland et al., 2010). Possible cause of the variation has been linked to the extended period (6-8 weeks) of avocado fruit flowering (Salazar-Garcia et al., 1998).

The MC, or its reciprocal, DM, is a simple maturity parameter to determine in avocado fruit compared with oil content and is currently the global avocado industry standard for measuring maturity of the fruit (Obenland et al., 2010). South African avocado fruit industries make use of MC to determine fruit maturity while other industries prefer the use of DM. However, pre-harvest, postharvest practises and environmental conditions affect the MC and DM (Blakey, 2011). Therefore when taking a representative fruit sample, it could have originated from a stressed tree or sun-exposed fruit to achieve a lower MC or a higher DM than the orchard mean, thereby creating a false impression of the orchard's readiness for harvest (Blakey, 2011). Alternative means that can give a reliable and fast indication of harvest maturity therefore need to be investigated.

Seven carbon (C7) sugars, *D*-mannoheptulose and perseitol, are dominant sugars in avocado fruit and have been suggested as likely indicators of avocado fruit maturity (Bertling and Bower, 2006; Blakey, 2011; Tesfay et al., 2012b). *D*-mannoheptulose, which is a major component of mesocarp total soluble solids (TSS), has been suspected to be responsible for the continued growth of the fruit (Cowan, 2004; Bertling et al., 2007). Determining total soluble solids (TSS) as an indicator of harvest maturity is a well-established method in horticultural products such as mango (Jha et al., 2006; Watanawan et al., 2014), grape (Guelfat-Reich and Safran, 1971; Jayasena and Cameron, 2008) and muskmelon (Wyllie et al., 1996). This harvest maturity indicator is a quick, simple, easy and objective measurement of maturity, generally expressed as °Brix (1% sucrose in a solution) (Jayasena and Cameron, 2008). It determines the soluble sugar and acids or solids in a given fruit (Eshed and Zamir, 1995). The potential use of TSS as an alternative indicator capable of providing reliable measurement of harvest maturity of avocado fruit was, therefore, investigated in this study.

2. Materials and methods

2.1. Fruit material

The research was carried out in 2013 and 2014 growing season using ‘Fuerte’ and ‘Hass’ avocado fruit. During 2013 season, avocado fruit were sampled from Bounty Farm (Latitude: 29°28´S; Longitude: 30°161´E). Samples were collected on 25 April, 17 July, 20 September, and 11 November, corresponding to early, mid and late harvesting period. On each harvesting day, a total of 200 fruit (100 per cultivar) were harvested randomly from 20 trees for the experiment. In 2014 season, another orchard, Everdon Estate (Latitude: 29°45’S, Longitude: 30°25’E), was included in the experiment in order to validate/ascertain the 2013 season experimental results. 'Fuerte’ and ‘Hass’ were harvested at two weeks interval from 14/02/2014 (golf ball size) to 20/03/2014 and then monthly from 20/03/2014 till 04/08/2014 (after commercial maturity). The two orchards are both located in the cool subtropical area of KwaZulu-Natal, South Africa. During each sampling date, 40 ‘Fuerte’ and 40 ‘Hass’ fruit were harvested from each of the two farms. Two fruit of uniform sizes were harvested per tree from 20 mature trees at each location. Although both locations are cool subtropical areas, Everdon Estate was characteristically slightly cooler than Bounty Farm and trees are under irrigation. Bounty Farm is solely dependent on rainfall. To minimise physical damage, harvested fruit were put in crates and immediately transported in a well-ventilated vehicle to the postharvest laboratory in the University of KwaZulu-Natal, where fruit were then sorted for size uniformity.

2.2. Sample preparation

Two core samples (2.5 ml each) were taken along the equatorial region of the fruit using a 15 mm diameter cork-borer. Two core tissue samples were flash-frozen in liquid nitrogen, lyophilized and then stored at -20 °C for further analysis while the remaining core sample was squeezed using garlic press to extract liquid which was later subjected to analysis.

2.3. Chemical

Standard grade hexane was used for oil extraction and was either purchased from ACE, Southdale, Johannesburg, South Africa or SIGMA-ALDRICH, St Louis, MO, USA.

2.4. Measurement of respiration rate

Fruit respiration rate was measured within 34 hours after harvest. Respiration rate was determined by measuring the amount of carbon dioxide evolved using an infrared gas analyser (EGM-1, PP Systems, Hitchin, Hertfordshire, UK). Individual fruit was measured by incubating in 1 litre jars for 10 minutes as previously described by Van Rooyen and Bower (2003). The net respiration rate of the atmosphere in the jars per gram fruit mass was calculated by adjusting for ambient carbon dioxide in the jar, fruit volume, head space and fruit mass.

2.5. Determination of total soluble solids (TSS)

The TSS (°Brix) of freshly harvested fruit was determined according to Jha et al. (2006) with slight modifications. A garlic press was employed to extract the juice of the hard cored sample and TSS of the mesocarp was determined using a digital refractometer (PR-101 ATAGO, Topac Inc, Cohasset, USA).

2.6. Measurement of the mesocarp oil content

Mesocarp oil content was measured from ground freeze-dried (lyophilised) sample material and quantified using a method described by Meyer and Terry (2008), with slight modifications. Hexane (9.0 mL) was added to 300 mg of the lyophilised mesocarp tissue in test tube and placed into a sonic bath for 10 min. The sample was filtered under vacuum and another 6 mL hexane added to the test tube. This was left for 5 min and the tube emptied into the Buchner funnel. The 15 mL extract were dried using GeneVac® concentrator (SP Scientific, Genevac Limited, IPSWICH ENG.). The extracted oil was weighed and presented on a dry mass basis (DM).

2.7. Percentage mesocarp moisture content

The MC and DM were determined by measuring the weight difference of the sample taken from equatorial region of the fruit before and after freeze drying.

2.8. Statistical Analysis

Experiments were carried out using a completely randomized design (CRD). Data for the analytical determinations were subjected to analysis of variance using GenStat® 16th Edition (VSN International, Hemel Hempstead, UK) and means were separated by Duncan's multiple range test. Differences at $p \leq 0.05$ were considered to be significant.

3. Results

There were significant differences between production locations and harvest dates in both, ‘Fuerte’ and ‘Hass’ avocado (Figs. 4.1, 4.2, 4.3, 4.4, 4.5). The trends in MC (Figs. 4.1A, 4.2A, 4.3A and 4.5A) and diameter (Figs. 4.4D and 4.6D) were fairly consistent over the sampling periods. Oil content recorded for ‘Fuerte’ fruit from Bounty farm had no significant difference from 17/07/2013 and 20/09/2013 (Fig. 4.1C). The recorded values for the maturity parameter (oil content) in 2014 for ‘Fuerte’ (Fig. 4.3C) fruit from both production locations tended to be present at high concentration than that of ‘Hass’ (Fig. 4.5C) fruit but did not show consistent trends. In 2013, the oil content of ‘Hass’ avocado fruit from Bounty farm followed an inconsistent trend but a peak was recorded on 20/09/2013 (Fig. 4.2C). Similarly, the oil content of ‘Hass’ fruit from Bounty Farm and Everdon Estate followed an inconsistent trend in 2014 (Fig. 4.5C). The oil content of ‘Hass’ fruit was lower than that of ‘Fuerte’ at the initial stage of the fruit growth, but oil content of ‘Hass’ increased over sampling period.

All parameters from both cultivars showed highly significant differences over the sampling period ($p < 0.001$) (Tables 4.1 and 4.2). There were significant interactions between the harvest time and production location for MC, DM, oil content, respiration rate and fruit mass, while there was no interaction with fruit diameter (Table 4.3).

In both seasons, TSS values were high significantly different between harvesting periods for ‘Fuerte’ fruit ($p < 0.001$) (2013 and 2014) (Tables 4.1 and 4.2). In 2013, the TSS measured for ‘Fuerte’ fruit ranged from 5.09 to 10.29, it reached a peak on 17/07/2013 and then decreased over the last two harvests, with no significant difference from 20/09/2013 to 11/11/2013 (Fig. 4.1D). A significant difference ($p = 0.016$) between harvesting periods, reaching a peak in July, was recorded for 'Hass' fruit with TSS ranging from 6.18 to 7.82 during the 2013 season (Fig. 4.2D). Though did not follow consistent pattern, 'Fuerte' fruit reached a peak in June 2014 for fruit harvested from Bounty Farm with TSS ranging from 3.87 to 6.37 (Fig. 4.3D). Total soluble solids showed highly significant difference ($p < 0.001$) between the two production locations in 2014 (Fig. 4.3D). A peak was recorded earlier on 08/05/2014 for 'Fuerte' fruit harvested from Everdon Estate with TSS range from 3.87 to 5.65 (Fig. 4.3D). A highly significant difference ($p < 0.001$) was also determined between harvesting periods during 2014 season (Fig. 4.3D). The TSS ranged from 4.08 to 5.21 for 'Hass' fruit harvested from Bounty Farm and 3.32 to 6.02 for fruit from Everdon Farm. There was no significant difference ($p = 0.344$) in mesocarp TSS between production locations in 2014 (Table 4.2).

There were significant differences in terms of diameter between different stages of development and between production locations for both 'Fuerte' and 'Hass' avocados (Fig. 4.3). For both cultivars, a rapid increase in diameter was observed at the beginning of the sampling period and then followed by a linear growth. 'Fuerte' fruit from Bounty Farm had a continuous growth trend exhibited by a significant increase in diameter as observed during the last stage of data collection.

3.1 Respiration rate

Though was not measured in 2013, there were highly significant differences ($p < 0.001$) in respiration rate of 'Fuerte' fruit harvested at different dates during 2014 growing season (Fig. 4.6B). Similarly, the effect of production on respiration rate measured fruit was significant ($p < 0.001$) over the sampling period. There were also significance differences between production locations for 'Hass' fruit ($p = 0.033$). Analysis of variance carried out for respiration rate of 'Fuerte' showed a significant interaction between harvest time and production locations. Significant differences occurred in the interaction between harvest periods and production locations for 'Fuerte' fruit ($p = 0.009$). This indicated that although the effect of orchard location on respiration rate was significant, it was also depended on harvest time. No significant interaction existed for 'Hass' fruit ($p = 0.478$).

3.2 Mesocarp moisture content

Harvest date significantly affected the MC in both cultivars harvested from Bounty Farm and Everdon Estate ($p < 0.001$) in 2013 and 2014 growing seasons (Tables 4.1 and 4.2), but there were no significant difference in the interaction between harvest date and production location for 'Hass' fruit ($p = 0.085$) in harvested in 2014 growing season. A consistent decrease in MC occurred from 20/03/2014 throughout the remaining harvest dates for 'Hass' fruit from both production locations.

3.3 Correlation between parameters

In ‘Fuerte’, the correlation factors (r_s) between MC, oil content, fruit mass and diameter was significant ($P < 0.001$) in both production locations, but the correlation between MC and diameter was weak ($r_s = -0.3604$) (Table 4.3). There were strong correlations between DM and oil content ($r_s = 0.78$) and between oil content and fruit mass ($r_s = 0.79$). Very weak correlations were recorded between diameter and oil content, as well as between diameter and fruit mass (r_s =0.09, -0.04, respectively; Table 4.3). In ‘Hass’ fruit, however, DM, as expected, was negatively correlated with MC as displayed in a very strong r_s of -0.99 as well as r_s of -0.81 and -0.77 with oil content and fruit mass respectively (Table 4.3). Weak correlations occurred between diameter and MC and oil content (r_s = -0.42, 0.36, respectively) (Table 4.3)

4. Discussion

4.1. Respiration

Fruit respiration rate of the fruit involves the oxidative disintegration of organic materials deposited (energy reserve) in the tissues. Although the determination of the climacteric ripening of the fruit was beyond the scope of this study, the rate at which harvested fruit respired within 24 hours after detachment from the tree was measured and found to generally increase with maturity for both cultivars from both locations. A relatively high rate of respiration occurred for ‘Fuerte’ fruit harvested from Everdon Estate on 03/06/2014 (Fig. 4.4B and Fig. 4.6B). This could be attributed to a relatively large sized fruit used for the measurements at this time.

4.2. Harvest maturity indicators of avocado fruit

Appropriate maturity of horticultural fruit at the point of harvest is critical to postharvest management of fruit as well as to deliver fruit of acceptable quality to consumers. The phase during growth and development at which a fruit is harvested affects palatability (Kader et al., 1985), vulnerability to mechanical damages, flavour components, ability to resist pathogen invasion (Shewfelt et al., 1987), ability to ripen to a desirable product (Crisosto et al., 2003) and postharvest handling (Kader, 1999). Due to different morphological, physiological and genetic make-up of horticultural crops, indices suggesting optimum maturity of fruit differ. Harvest maturity of avocado fruit is difficult to determine because objective indices, such as colour, size and shape, fresh mass, and firmness, do not effectively suggest maturity of the fruit. However, these indices are capable of determining the harvest maturity of fruit such as stone fruit (Romani and Jennings, 1971; Stembridge et al., 1972), grapefruit, and tomato (Ryall and Pentzer, 1982).

Indicators capable of suggesting minimum maturity of avocado fruit are subjective and include MC, DM and oil content. Therefore, during the development of ‘Fuerte’ and ‘Hass’ avocado fruit in the current study, there was negative relationship between MC and oil content. A close relationship between the increase in oil content and the decrease in MC during fruit growth and development (Appleman and Noda, 1941; Hall et al., 1955; Slater et al., 1975; Lee, 1981; Kruger et al., 1995). The MC linearly decrease (Figs. 4.4A and 4.5A) over the harvesting time from February to August 2014, in agreement with a previous study conducted by Kruger et al. (2014). The oil content generally increased over time (Figs. 4.4C and 4.5C). The decrease in MC in both cultivars followed a consistent trend relative to the oil content which increased inconsistently in both cultivars. In view of this, the MC seems to be a more precise parameter reflecting the maturity of avocado fruit; however, as no peak in MC was observed, the continuous decrease in MC could not indicate the stage at which the fruit should be harvested. Moriss and O'Brien (1980) suggested that the point during the decrease in MC at which the seed coat of the fruit is dark and dry could be associated with avocado fruit maturity, thereby suggesting a point of harvest. Since commercial or horticultural maturity implies physiological maturity of avocado fruit, it would be of scientific benefit to investigate and establish, if the point at which the seed coat of the fruit dies as represented by the dark colour (Morre-Gordon et al., 1995) is associated with physiological maturity of avocado fruit. Further, there was no significant difference between the two production locations from February to 04/04/2014 with respect to MC of 'Fuerte' fruit (Fig. 4.3A). This could be associated with the cool climatic condition of both farms (cool subtropical area). The MC of 'Hass' avocado from both production locations showed no significant difference throughout the entire experimental period; however, there were significant differences between production locations for oil content (Fig. 4.5A and 4.5C, respectively). The oil content for 'Hass' fruit from both farms had the highest value of 30.5% oil content for fruit from Bounty Farm and 27.7% oil content for those from Everdon Estate on 04/08/2014 when the final samples were collected (Fig. 4.5C). At this stage, fruit from both farms were well above the minimum oil content of 8% recommended before avocado fruit could be harvested for commercial purposes (Kruger et al., 1995). The oil content of the early maturing cultivar, 'Fuerte', from Everdon Estate reached a peak with the value 26.9% oil content on 08/05/2014 while oil content for 'Fuerte' fruit from Bounty Farm had the highest percentage at the last day of harvest with 24.9% oil content (Fig. 4.3C). This shows that fruit from Everdon Estate matured faster those from Bounty Farm which could be as a result of cultural practices (Raquejo-Tapia et al., 1999).

4.3 Total soluble solids

The determination of harvest maturity of horticultural fruit crops is of paramount importance to postharvest quality. Since avocado fruit lack physical characteristics that suggest harvest maturity, an internal component that could be measured easily and provide such an indication would, therefore, be desirable. This quest for an easily determinable parameter has led to study the internal composition of avocado fruit during development in order to be able to identify a harvest maturity indicator (Mougheith et al., 1978). Focusing on the mesocarp of the fruit, it has been reported that the actively dividing mesocarp cells of the fruit accumulate carbohydrates, proteins (Davenport and Ellis, 1959; Mougheith and Adel-Hmid, 1978) and fatty acids (Mougheith and Adel-Hmid, 1978; Eaks, 1990). Similarly, C7 sugars were suspected to be responsible for the continued growth of avocado fruit (Cowan, 2004; Bertling and Bower, 2005; Bertling et al., 2007) as well as possible indicators of harvest maturity (Bertling and Bower, 2006; Blakey, 2011; Tesfay et al., 2012b). Therefore, it was expected that TSS would accumulate as fruit grows (Bertling and Bower, 2006). However, the result of this study showed no consistent trend in TSS for both avocado fruit cultivar experimented in both seasons and therefore no relationship could be said to exist with the increasing sugar as reported by Blakey (2011) and Tesfay et al. (2012b). Similarly, no relationship could be established between TSS (Figs. 4.1D and 4.2D) and the fruit growth measured in diameter (Figs. 4.4A and 4.6A), respectively. The high TSS observed in fruit such as kiwifruit (Gorini et al., 1990), cherries (Crisosto et al., 2003), peaches (Robertson et al., 1988) and certain grapes cultivars (Sonego et al., 2002) is an indication of maturity and most of the solids in these fruit are soluble sugars. These sugars increase as the fruit matures, however, the overall low range of TSS recorded in this experiment (Figs. 4.1D, 4.2D, 4.3D and 4.5D) showed no indication of maturity in avocado fruit.

5. Conclusion

Overall, the results obtained in the study to evaluate TSS as an alternative indicator of maturity of ‘Fuerte’ and ‘Hass’ avocado fruit were inconsistent in both seasons. Therefore, it can be concluded that TSS cannot be used as an indicator of avocado fruit maturity. However, MC appeared to be a better indicator for determining avocado fruit maturity as suggested in previous studies.

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Table 4.1: P-values for analysis of variance for harvest time for ‘Fuerte’ and ‘Hass’ avocado fruit during the 2013 growing season from Bounty Farm in the cool subtropical area of KwaZulu-Natal, South Africa.

		P-value				
Cultivar	Factor	DM (%)	MC (%)	Oil content (%)	TSS (°Brix)	Fruit Mass (g)
Fuerte	Harvest Time	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Hass	Harvest Time	< 0.001	< 0.001	< 0.001	0.016	< 0.001

Table 4.2: P-values for analysis of variance for harvest time, production location and the interaction between harvest time and production location for ‘Fuerte’ and ‘Hass’ avocado fruit harvested from February 2014 to September 2014 from two commercial orchards in the cool subtropical area of KwaZulu-Natal, South Africa.

		P-value						
Cultivar	Factor	DM (%)	MC (%)	Oil content (%)	TSS (°Brix)	Diameter (mm)	Respiration (mL CO_2/kg fruit/hr)	Fruit Mass (g)
Fuerte	Harvest Time	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Production location	<0.001	<0.001	0.012	<0.001	<0.001	<0.001	<0.001
	Harvest Time. Production location	<0.001	<0.001	<0.001	<0.001	0.840	0.009	<0.001
Hass	Harvest Time	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Production location	0.030	0.025	0.723	0.344	<0.001	0.033	<0.001
	Harvest Time. Production location	0.091	0.085	<0.001	<0.001	0.314	0.478	<0.001

Table 4.3: Correlation coefficients between, MC, oil content, fruit mass, diameter and DM of 'Fuerte' and 'Hass' avocados during the 2014 growing season. Values are from fruit that were harvested from two commercial orchards in the cool subtropical area of KwaZulu-Natal, South Africa.

Cultivar	Parameter	MC (%)	Oil content (%)	Fruit Mass (g)	Diameter (mm)	DM (%)
Fuerte	MC (%)	-				
	Oil content (%)	0.5147	-			
	Fruit Mass (g)	0.6650	0.7937	-		
	Diameter (mm)	-0.3604	0.0932	-0.0416	-	
	DM (%)	0.4525	0.7759	0.7949	0.1609	-
Hass	MC (%)	-				
	Oil content (%)	-0.8128	-			
	Fruit Mass (g)	-0.7684	0.6829	-		
	Diameter (mm)	-0.4210	0.3585	0.5610	-	
	DM (%)	-0.9996	0.8115	0.7671	0.4203	-

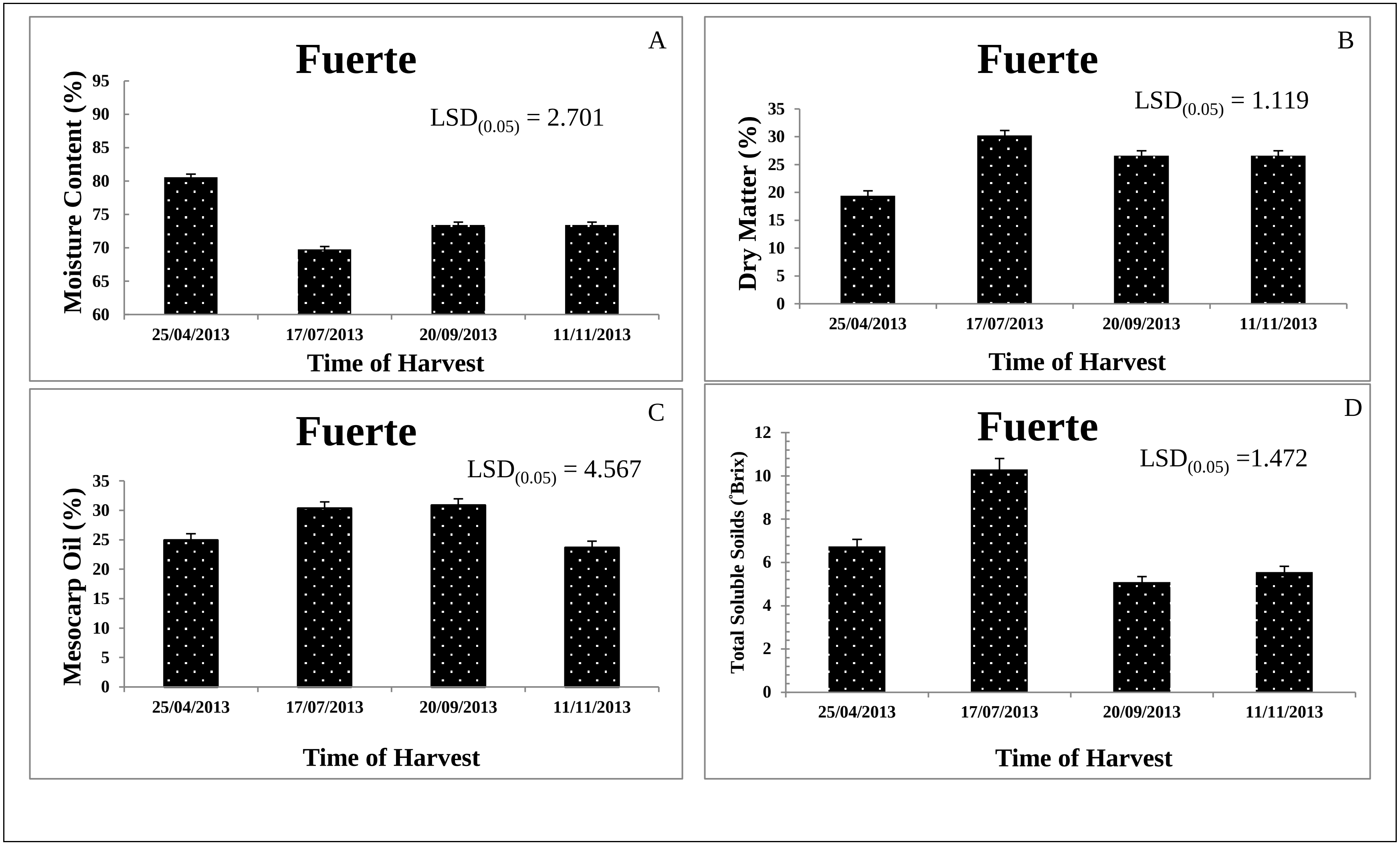

Fig. 4.1: Changes in moisture content (A), dry matter (B), oil content (C) and total soluble solids (D) of ‘Fuerte’ avocado from Bounty farm in the cool subtropical area of KwaZulu-Natal, South Africa during the 2013 growing season.

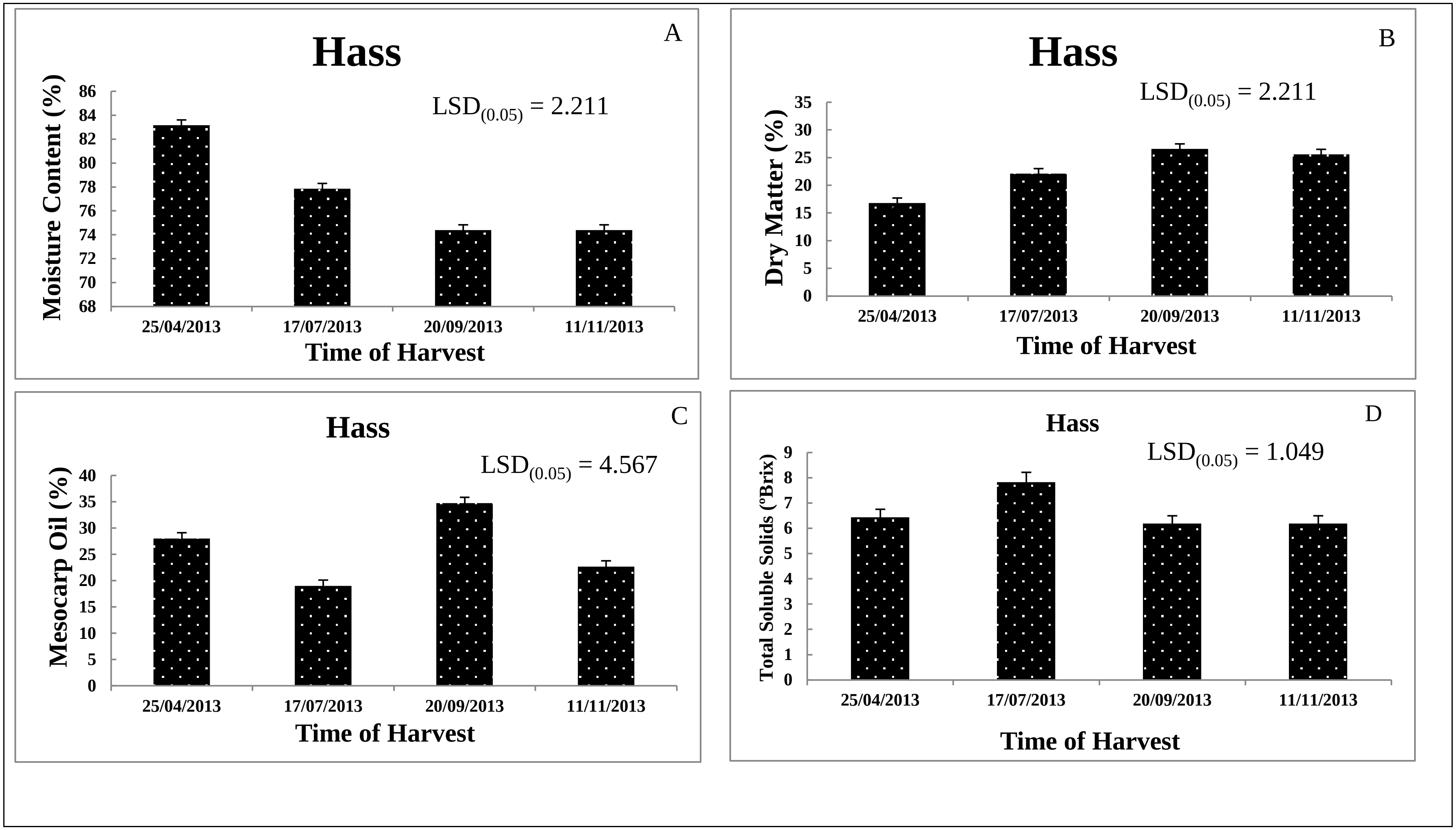

Fig. 4.2: Changes in moisture content (A), dry matter (B), oil content (C) and total soluble solids (D) of 'Hass' avocado from Bounty farm in the cool subtropical area of KwaZulu-Natal, South Africa during the 2013 growing season.

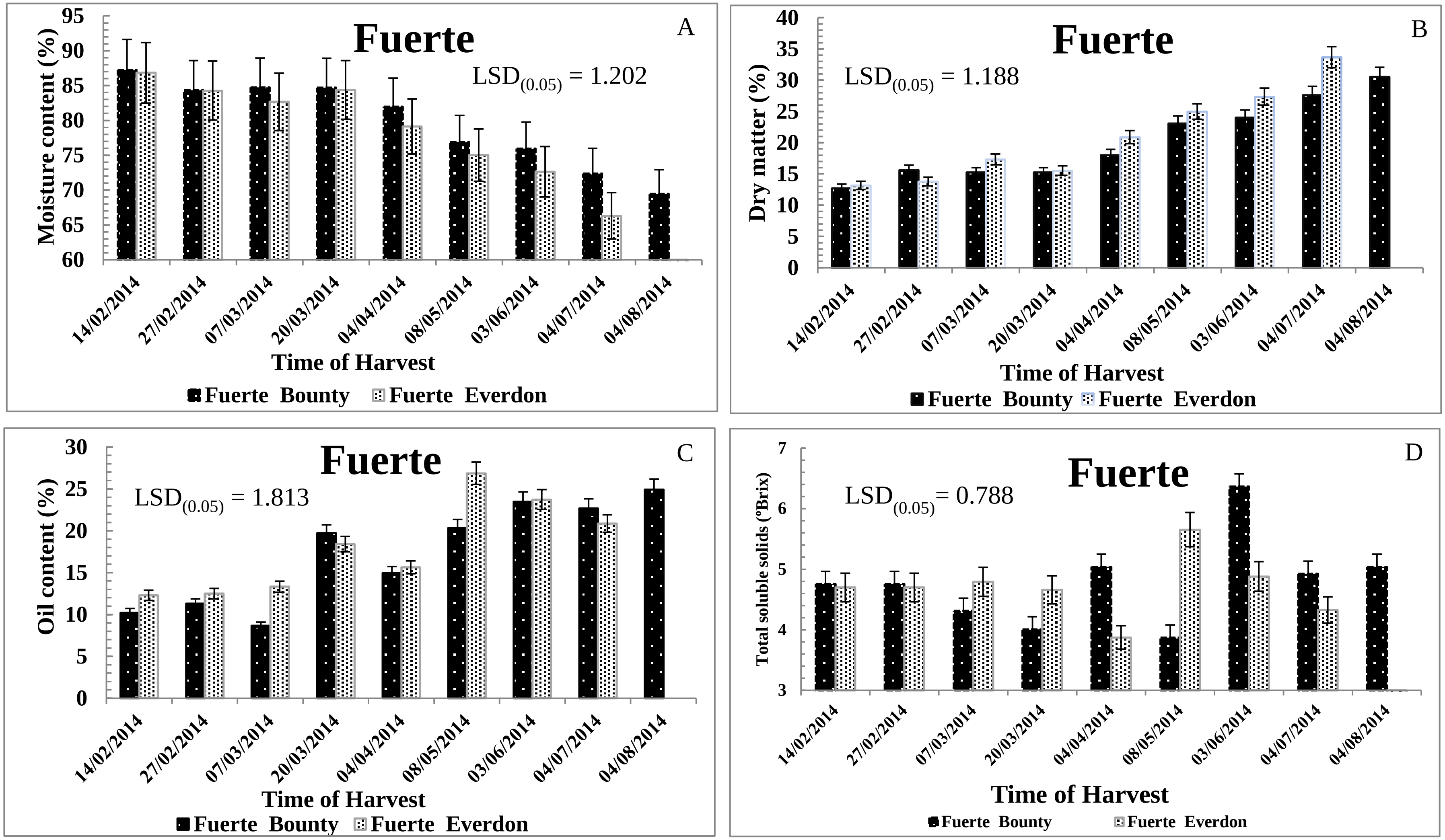

Fig. 4.3: Changes in moisture content (A), dry matter (B), oil content (C) and total soluble solids (D) of 'Fuerte' avocado from two commercial production locations in the cool subtropical area of KwaZulu-Natal, South Africa during the 2014 growing season.

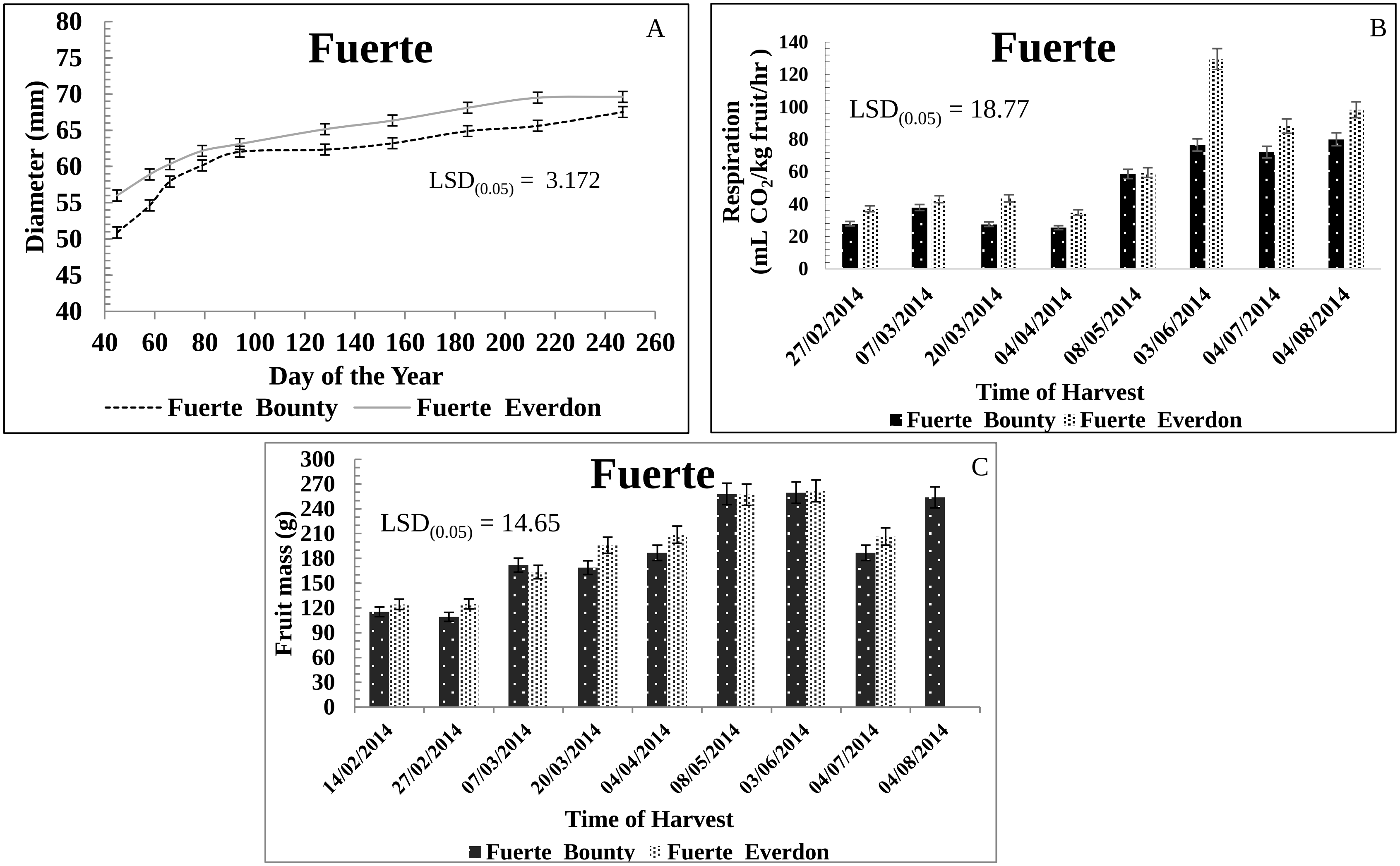

Fig. 4.4: Changes in diameter (A), respiration (B) and fruit mass (C) of ‘Fuerte’ avocado from two commercial production locations in the cool subtropical area of KwaZulu-Natal, South Africa during the 2014 growing season.

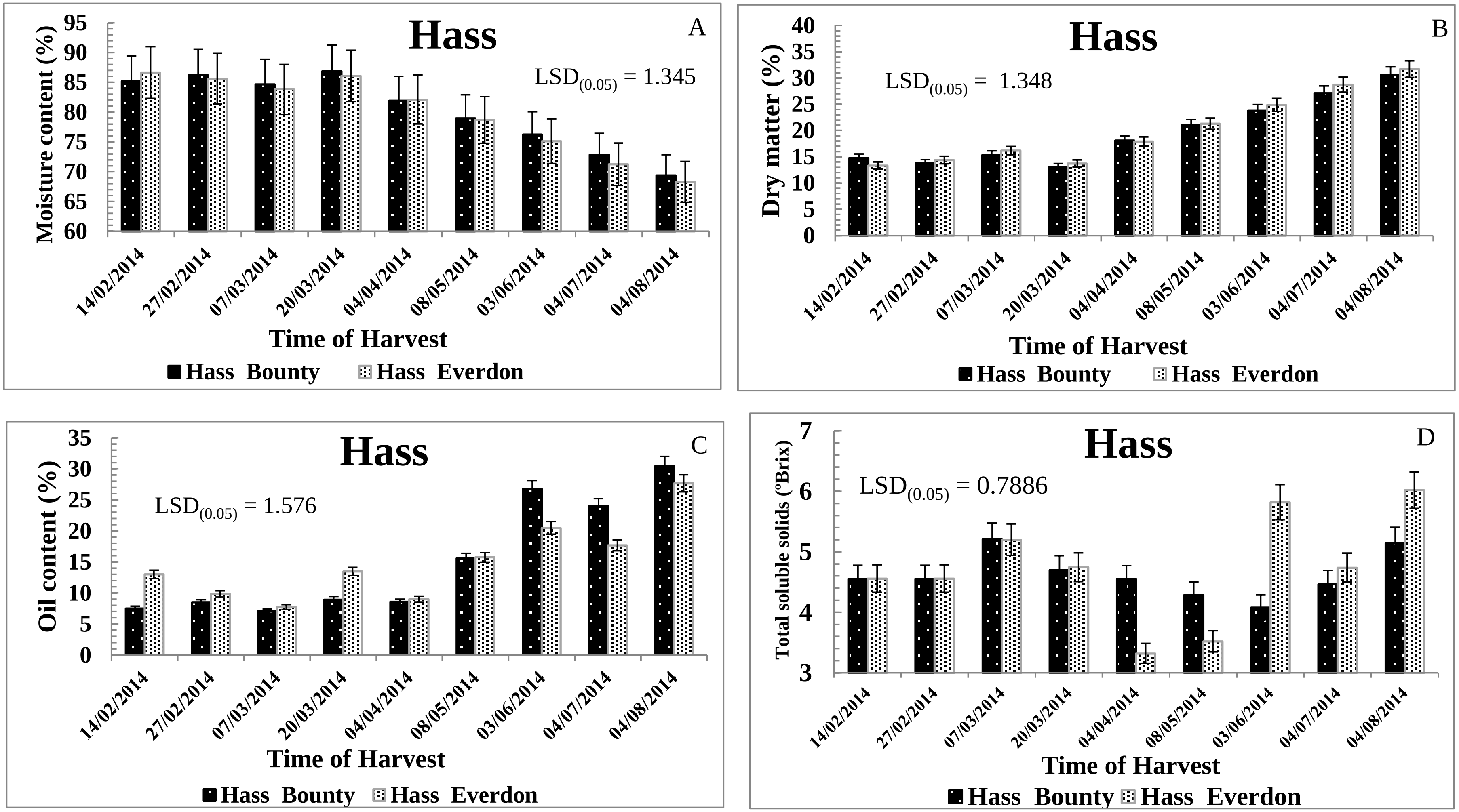

Fig. 4.5: Changes in moisture content (A), dry matter (B), oil content (C) and total soluble solids (D) of ‘Hass’ avocado from two commercial production locations in the cool subtropical area of KwaZulu-Natal, South Africa during the 2014 growing season.

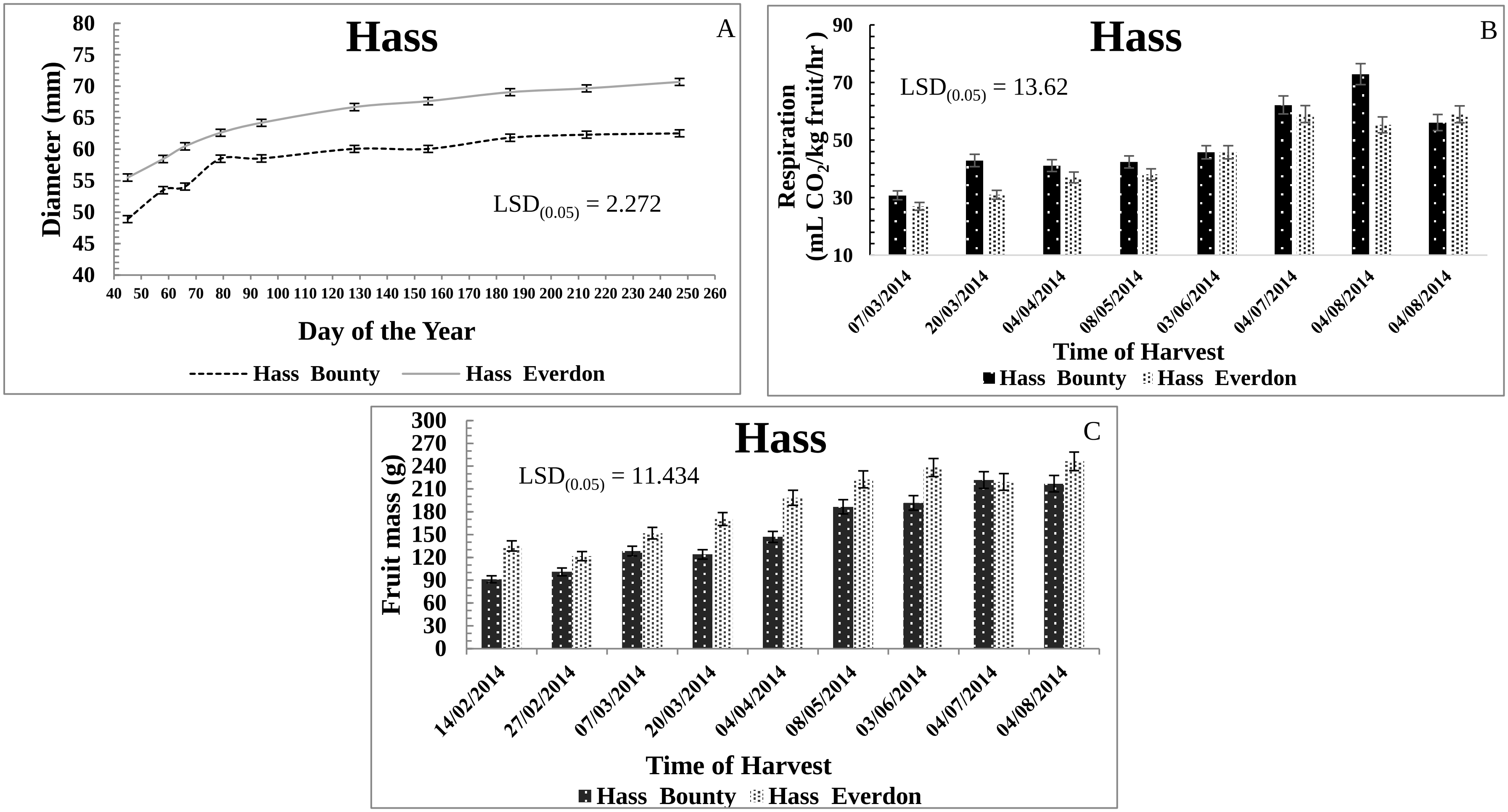

Fig. 4.6: Changes in diameter (A), respiration (B) and fruit mass (C) of ‘Hass’ avocado from two commercial production locations in the cool subtropical area of KwaZulu-Natal, South Africa during the 2014 growing season.

CHAPTER 5

DEVELOPING ROBUST NIRS-BASED NON-DESTRUCTIVE METHODS FOR EVALUATING MATURITY PARAMETERS OF 'HASS' AVOCADO FRUIT

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Abstract

The feasibility of near-infrared spectroscopy (NIRS), as a rapid non-destructive method for predicting maturity parameters of ‘Hass’ avocado fruit, was evaluated. A total of 155 intact ‘Hass’ avocado fruit were harvested and scanned in reflectance mode of the NIRS, during 2013 and 2014 growing season. Reference maturity parameters, including percentage mesocarp moisture content (MC), percentage dry matter (DM) and oil content were measured using conventional destructive methods. The acquired spectral data was subjected to principal component analysis (PCA), to identify spectra outliers. A chemometric analytical tool, partial least square (PLS) regression was applied to the spectral data to develop prediction models for each of the parameters. Sixty percent of the spectra and destructive dataset used for model calibration and the remaining forty percent was used for validation. For external validation, PLS models developed during 2013 season were used to predict dataset acquired during 2014. NIRS prediction results showed that MC and DM were predicted with high accuracy having residual predictive deviation (RPD) of 2.00 and 2.13, respectively. However, models for predicting oil content were not accurate, with RPD value 1.0. The good prediction statistics between NIRS predicted MC and DM content demonstrated the potential of this system for non-destructive evaluation of avocado fruit maturity parameters. The high prediction accuracy observed when models developed in 2013 were used to predict maturity of fruit harvested during 2014 season demonstrated robustness of PLS models. Where speed and accuracy are required for assessing maturity status of individual intact avocado fruit, the method developed in this study is recommended.

Keywords: Avocado, ‘Hass’, maturity, near infrared spectroscopy, non-destructive technique, oil content

1. Introduction

Evaluation of maturity status of avocado fruit in a consignment is currently based on destructive measurements. This is unlike most other horticultural products whose maturity parameters can be based on external characteristics such as size, shape and colour (Blasco et al., 2003; Cubero et al., 2010; Kondo, 2010). Destructive measurements usually involve few representative samples on which the maturity of other individual avocado fruit is based (Blakey, 2011). The disadvantage of this method, apart from its labour intensive and destructive nature, is that growers stand the risk of delivering poor quality fruit to the market due to fruit quality variation within a batch. Poor quality fruit resulting from immature fruit, may lead to consumer dissatisfaction and affect future purchase of the fruit.

Avocado fruit do not exhibit external changes during maturation. Hence, internal biochemical parameters such as mesocarp moisture content (MC), dry matter (DM) and oil content are used as indicators of maturity status. Several studies have been conducted in recent years to evaluate and develop near infrared (NIR) spectroscopy (NIRS) for non-destructive assessment of avocado maturity parameters (Blakey and van Rooyen, 2011; Wedding et al., 2013). However, very limited or no research has been conducted to demonstrate the effectiveness of NIRS to predict oil content which is considered the most reliable parameter for determining avocado fruit maturity (Lee et al., 1983; Kaiser, 1994).

The NIRS has been revealed as a precise, rapid and non-destructive alternative to destructive methods for providing non-visible information about comparative proportions of C-H, O-H and N-H bonds (Wetzel, 1983; Williams and Norris, 1987; Shenk et al., 2001; Workman and Shenk, 2004; Nicolaï et al., 2007; Magwaza et al., 2012). In view of this, analyzing the absorption spectra would aid the determination of maturity parameters of avocado fruit including the oil content.

Chemometrics is an important aspect of NIRS as it involves multivariate analysis for the interpretation of the large data sets of NIR spectra (Wetzel, 1983; Wang and Paliwal, 2007). Therefore, calibration model must be developed by calibrating the NIR spectra against the destructive (reference) method (Magwaza et al., 2012). This model can be developed using various chemometric tools such as partial least square regression (PLS), multivariate linear regression (MLR) and principal component regression (PCR) (Nicolaï et al., 2007). PLS is mostly preferred for the development of calibration models because of its ability to remove latent variables (LVs) that are not important to describe the variance of maturity parameter (De Jong, 1993). This current research aims to evaluate the use of NIRS in reflectance mode to predict the maturity parameters of intact 'Hass' avocado fruit.

2. Materials and methods

2.1 Avocado fruit sample selection

The research was carried out during 2013 and 2014 seasons using 'Hass' fruit harvested from a commercial farm (Bounty farm) located in the cool subtropical area of KwaZulu-Natal, South Africa (Latitude: 29°28´S; Longitude 30°161´E). During 2013 season, 100 fruit were harvested randomly from 20 trees for both destructive and non-destructive measurements during the early (25 April), mid (17 July) and late harvesting (20 September and 11

November) periods. However, fruit harvested during the mid and late harvesting periods could not be scanned by the NIRS due to technical fault of the equipment. Other sets of fruit were harvested in 2014 season from another commercial farm (Everdon Estate) from the same cool subtropical area of KwaZulu-Natal, South Africa (Latitude: 29°45’S, Longitude: 30°25’E) was incorporated for external validation. During 2014, fruit were harvested from February to August 2014, and a total of 80 fruit were picked randomly on every harvest date from the two farms. Two fruit per tree were harvested from 20 trees from each orchard. Harvested fruit were put in crates and immediately transported in a well-ventilated vehicle to the postharvest laboratory at the University of KwaZulu-Natal. Fruit were then sorted for uniformity of size and defects and allowed to equilibrate at room temperature before scanning.

2.2 NIR spectra acquisition

The NIR spectra of intact ‘Hass’ avocado fruit sample were acquired using a method described by Wedding et al. (2013) with modifications. Briefly, spectral data was measured in reflectance mode using a bench-top fibre-optic spectrophotometer (Perstorp Analytical, FOSS NIRSystem®, Inc., USA) operated with Vision software (Vision^{TM}, version 3.5.0.0) in the 400-2500 nm range.

2.3 Destructive (laboratory reference) analysis

Reference destructive measurements were taken from an area of the fruit where the spectra were acquired. Destructive measurements of the fruit for maturity parameters, including MC, DM and oil content were carried out on each avocado fruit sample. Two cores were taken from the marked area of the fruit around the equator using a 15 mm diameter steel corer. The exocarp, seed coat and the seed were removed and mesocarp sample was snap frozen in liquid nitrogen, lyophilized and then stored at -20 °C in a -20 °C for further analysis.

2.3.1 Percentage mesocarp moisture content

The MC and DM were determined by measuring the weight difference of the sample taken from equatorial region of the fruit before and after freeze drying.

2.3.2 Mesocarp oil content

The mesocarp oil was measured from the ground sample material and was quantified using a method described by Meyer and Terry (2008), with slight modifications. Hexane (9.0 mL) was added to 300 mg mesocarp and the test tubes placed into an ultrasonic bath for 10 min. The supernatant was filtered under vacuum and another 6 mL hexane added to the sample test tube. This was left for 5 min and the tube emptied into the Buchner funnel. The 15 ml of hexane was combined and dried using a GenVac® concentrator (SP Scientific, Genevac Limited, IPSWICH ENG.). The oil was weighed and presented on dry weight basis.

2.4 Data analysis

Chemometric analyses such as principal component analysis (PCA) and partial least squares (PLS) were done using the Unscrambler software (The Unscrambler$^{\text{TM}}$ Version 10.1, CAMO, Oslo, Norway).

Spectral data were subjected to various pre-processing methods to correct light scattering and reduce the changes of light path length (Magwaza et al., 2012). Pre-processing methods studied included: smoothing using moving average, Gaussian and median filters and Savitzky-Golay methods, multiplicative scatter correction (MSC), Norris Gap, Gap-segment and Savitzky-Golay first and second derivative, standard normal variate (SNV), area, maximum, unit vector and mean normalization, and orthogonal signal correction methods. The combination SNV transformation and 2-point SG first derivative (2nd order polynomial) spectral smoothing gave more stable models and were selected for model prediction (Fig. 5.2). The SNV was used to remove multiplicative interferences of scatter and particle size (Barnes et al., 1989) while the SG first derivative was used to remove the baseline trend of the spectra (Rinnan et al., 2009).

Noise was noticed at the margins of the spectra range collected and for purposes of getting good modelling results; the spectra were truncated to a range of 800 – 2400 nm before model development. The spectral variation, outliers and effectiveness of the spectra were investigated by PCA using full cross validation. As suggested by Kuang and Mouazen (2011), detected outliers which are far from the zero line of the residual line of the residual variance plot were removed from the calibration model. Prediction models were developed for MC, DM and oil content by applying PLS regression to the spectral data after pre-processing. Prior to the model development, the dataset were grouped into various calibration/training and prediction/test sets. These include calibration (60) and prediction (40) sets for the 2013 early season data. The developed model performances were based on the regression statistics described by correlation (Corr.), coefficient of determination (R^2) of the calibration (R_c^2) and prediction/validation (R_p^2), root mean square error of cross validation (RMSECV), root mean square error of prediction (RMSEP) in relation to the bias which is the average difference between the predicted and the reference data and the residual predictive deviation (RPD). RPD is the ratio of the standard error of prediction to the standard deviation (Williams and Norris, 1987). A good predictive model is considered to have (R_p^2) and RPD values of high percentage as well as low RMSECV and RMSEP values. The reliability of the model was verified by randomly substituting calibration and prediction data sets and examining that the differences in the regression statistics achieved were minimal (Alvarez-Guerra et al., 2010).

3. Results and discussion

The spectral variables which made significant contribution to the calibration and prediction model were explicated from the regression coefficients curve. Spectra range with high regression coefficients means that the variables were significant to the model while the regression coefficients close to zero were not important in the model development. Fig. 5.3 shows the regression coefficients of MC (A), DM (B) and oil content (C) models developed using spectra acquired from intact 'Hass' avocado harvested in 2013 and 2014 growing seasons. The graph (Fig. 5.3) symbolises the average spectra of 80 fruit randomly selected from the two seasons and used after applying SNV and SG first derivative pre-processing methods for calibration. The conspicuous peaks of the spectra as illustrated in the graph are at 1096, 1098, 1100, 1102, 1184, 1476, 1518, 2306, 2318 and 2346 nm. Spectra co-linearity habitually makes interpretation of NIR models in relation to various fruit components challenging. This is because the information obtainable in a particular calibration model could be carried by pooled effect of several wavelengths with each contributing fairly little information to the model and not necessarily by few independent wavelengths (McGlone and Kawano, 1998). Therefore, better prediction could be achieved if the whole NIR regions are utilised during calibration model development than using a few segments of the NIR or individual wavelength positions (Ozaki and Christy, 2007). The peak at 1184 (Fig. 5.3) are closely associated with the H-O-H stretching modes of water (Lestander and Geladi, 2005; Clément et al., 2008). The wavelength bands between 900-920 nm, around 930, 1200, 1750 and between 2200-2400 nm were associated with the C-H_2 stretching and combinations related to oil (Osborne and Künnemeyer, 1999; Clark et al., 2003; Guthrie et al., 2004). Strong and high-pitched wavelength bands are particularly noted between 2300-2400 nm as reported by Man and Moh (1998). Similarly, wavelength bands within the range 1300-1750 nm provide useful information for the determination of oil (Williams and Norris, 1987; Delwiche and Massie, 1996). Clark et al. (2003) reported that the 900-920 nm NIR wavelength bands is mostly essential for determination of DM and possibly sugar. The short-wavelength NIR region 700-1100 nm have been reported to permit better penetration into biological material while the long-wavelength region (above 1100 nm) have limited penetration but provide information on biological material that are rather close to the surface of a measured sample (Guthrie et al., 2004; Saranwong et al., 2004; Wedding et al., 2012). Based on the regression coefficient results in this study, useful wavebands for MC, DM and oil content were between 800-2400 nm. These findings are in line with the interpretation of wavelength bands of OH and CH stretching and combinations (Delwiche and Massie, 1996; Man and Moh, 1998).

Table 5.1 displays the summary statistics for the calibration and prediction models statistics for the prediction of measured maturity parameters of intact ‘Hass’ avocado. After critically analysing different ranges of wavelength based on the peaks illustrated in Fig. 5.3 and available information presented in literature, wavelength ranges that gave the best Corr., R^2, least RMSEC, RMSEP and RPD were chosen for the development of model for each maturity parameter. As stated earlier, the models for the maturity parameters were developed using the NIR range 800-2400 nm. This is in agreement with the range used in previous studies to develop models for the predicting DM of ‘Hass’ avocado (Workman and Shenk, 2004; Wedding et al., 2011b; Wedding et al., 2012; Wedding et al., 2013).

PLS calibration models developed for predicting maturity parameters of avocado fruit such as MC, DM and oil content for each season (early harvest in 2013 and 2014 harvest) showed good and poor performances, with respective predictive R^2 values of 0.80, 0.80 and 0.70 for 2013 season and 0.80, 0.80 and 0.42 for 2014 season and corresponding RMSEP of 3.13, 3.13 and 5.01 during 2013 season and 2.20, 2.20 and 6.00 in 2014 season (Table 5.1, Fig. 5.4) These results were in agreement with previous values reported in literature by Blakey et al. (2008) and Blakey and van Rooyen (2011) for the prediction of MC as well as those reported for the prediction of DM by Wedding et al. (2012) and Wedding et al. (2013).

Peirs et al. (2003) suggested that since spectral variations which occur as a result of biological variability of prospective samples are difficult to predict then seasonal effects have an extensive impact on calibration models for horticultural product. The impact of variability induced by seasonal effects was evaluated in this study for the fruit harvested from the cool subtropical area of KwaZulu-Natal, South Africa over the two seasons. To perform this evaluation, a PLS calibration model was developed using spectra from the 2013 season dataset to predict the maturity parameters of ‘Hass’ avocado for 2014 season population. Furthermore, a calibration model involving randomly selected dataset from the two seasons was developed to predict the other dataset selected for prediction purpose. The summary statistics for calibration and prediction models for these combinations are displayed in Table 5.2.

The results showed better PLS calibration model using 2013 season dataset for both MC and DM with R_c^2 -values of 0.91 and corresponding RMSECV of 2.27. The resulting model for oil content was fair with R_c^2 -value of 0.53 and RMSECV of 5.16. However, the application of these models to predict data population of 2014 growing season was not very effective (Table 5.2). These results explicated the impact of seasonal effect on biological materials as these materials vary greatly over different seasons (Peirs et al., 2003; Wedding et al., 2012). On the other hand, the calibration model which involved the random selection of data from the two seasons showed a significantly better performance as more biological variability was included in the models, with predictive R_p^2-values of 0.84 for both MC and DM, and 0.58 for oil content and corresponding RMSEP of 2.49 and 5.40, respectively (Fig. 5.5). These results were similar to the predictive performance of the model developed using more than one season in the calibration model to predict maturity parameter of avocado fruit (Wedding et al., 2010; Wedding et al., 2011a; Wedding et al., 2012) and internal quality of apple (Liu and Ying, 2005).

Different studies suggested that it is important to confirm the reliability of a model by checking its RPD values even though highly significant correlation exists between the NIR predicted and actual laboratory values (Saeys et al., 2005; Davey et al., 2009; Magwaza et al., 2012). RPD value < 1.5 means that the model is unreliable, those between 1.5 and 2.0 are appropriate for rough predictions, those between 2.0 and 2.5 are fit for quantitative predictions, those between 2.5 and 3.0 are considered good models while those > 3.0 are regarded as satisfactory models (Malley et al., 2000; Saeys et al., 2005; Zimmermann et al., 2007; Davey et al., 2009; Magwaza et al., 2012). The RPD values, 2.06 and 2.19 for both MC and DM respectively for 2013 individual season (Table 5.1) clearly indicate a fit model for quantitative prediction of the parameters while the RPD value 1.23 for oil content shows that the model is unreliable for prediction. The RPD value, 1.87 for both MC and DM for 2014 individual season (Table 5.1) shows that the model is appropriate for rough prediction of both MC and DM of ‘Hass’ avocado fruit. The RPD value, 1.07 for oil content indicates the unreliability of the model in providing accurate prediction of the maturity parameter but could be used as screening method (Malley et al., 2000).

Moreover, the maturity parameters had higher RPD for the models that involved random selection of data from the two seasons compared to the ones developed using 2013 early season dataset to predict 2014 season (Table 5.2). For the predictive model, MC and DM had similar RPD values of 0.85 and 0.84 while oil content had RPD of 0.40 (Table 5.2). These low values obviously indicate poor accuracy of the models. However, higher RPD values of 2.00 and 2.13 for MC and DM respectively and RPD value of 1.00 for oil content were recorded for predictive models developed from integrating the two seasons. The RPD values for MC and its reciprocal value, DM, indicated that the models were fit for quantitative prediction of the parameters in ‘Hass’ avocado fruit while the value for oil content clearly suggested that the model could not be trusted (Davey et al., 2009).

The unreliability of the developed model to predict oil content could possibly be because of the low concentration of oil in the fruit that were harvested during the early seasons of 2013 and 2014. The fruit had higher percentage of moisture which could have possibly masked or dominated the concentration of the available oil in the fruit and therefore limits the chances of NIRS of capturing higher peaks for the wavebands associated with the carbon hydrogen bonding related to oil. In future, it will be necessary to include samples from mid and late harvesting periods in a season. This is to guarantee a wider distribution of oil content concentrations to avoid the low predictability of the model.

4. Conclusion

Generally, the calibration and prediction model results in this current study confirmed that NIRSystem in reflectance mode predicted MC and the reciprocal value, DM of intact 'Hass' avocado fruit with a significant reliability. The results also demonstrated importance of integrating multi-seasonal dataset in a predictive model as it is capable of enhancing the predictive performance of the models. Further evaluation of the statistical results of the prediction models showed the potential of the technique to non-destructively predict oil content.

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Table 5.1: Summary of PLS calibration (cal.) and prediction (pred.) statistics for moisture content, dry matter and oil content for intact ‘Hass’ avocado fruit from KwaZulu-Natal, South Africa for 2013 and 2014 seasons.

Parameters	Season	Spectra	Range	Mean	SD	LV	R^2	RMSEC	RMSEP	SEP	Slope	Bias	Corr.	RPD
MC (%)	2014													
	Cal.	32	67.80-84.10	76.60	4.90	7	0.90	1.28			0.68			
	Pred.	23	69.60-83.70	77.60	4.10	7	0.80		2.20	2.90	0.78	1.03	0.92	1.87
	2013													
	Cal.	60	62.80-85.40	81.90	3.63	7	0.92	1.01			0.92			
	Pred.	40	56.70-84.70	79.34	6.80	7	0.80		3.13	3.30	0.87	0.50	0.92	2.06
DM (%)	2014													
	Cal.	32	15.40-33.7	22.70	5.69	7	0.95	1.28			0.95			
	Pred.	23	16.30-30.40	22.36	4.11	7	0.80		2.20	1.99	0.77	-1.03	0.92	1.87
	2013													
	Cal.	60	14.60-37.30	18.04	3.48	7	0.92	1.01			0.92			
	Pred.	40	15.30-43.30	20.70	6.80	7	0.80		3.13	3.13	0.87	-0.50	0.90	2.19
Oil content (%)	2014													
	Cal.	32	6.00-32.70	18.67	8.20	7	0.98	1.23			0.97			
	Pred.	23	6.90-29.40	16.30	6.40	7	0.42		6.00	5.78	0.57	-2.02	0.70	1.07
	2013													
	Cal.	60	13.70-35.6	18.65	3.61	6	0.55	3.28			0.55			
	Pred.	40	15.30-43.30	20.67	6.84	6	0.70		5.01	5.56	0.59	1.29	0.87	1.23

SD, Standard deviation; LV, Latent variable; R^2, coefficient of determination; RMSEC, Root mean square error of calibration; SEP, Standard error of prediction; Corr., Correlation; RPD, residual prediction deviation.

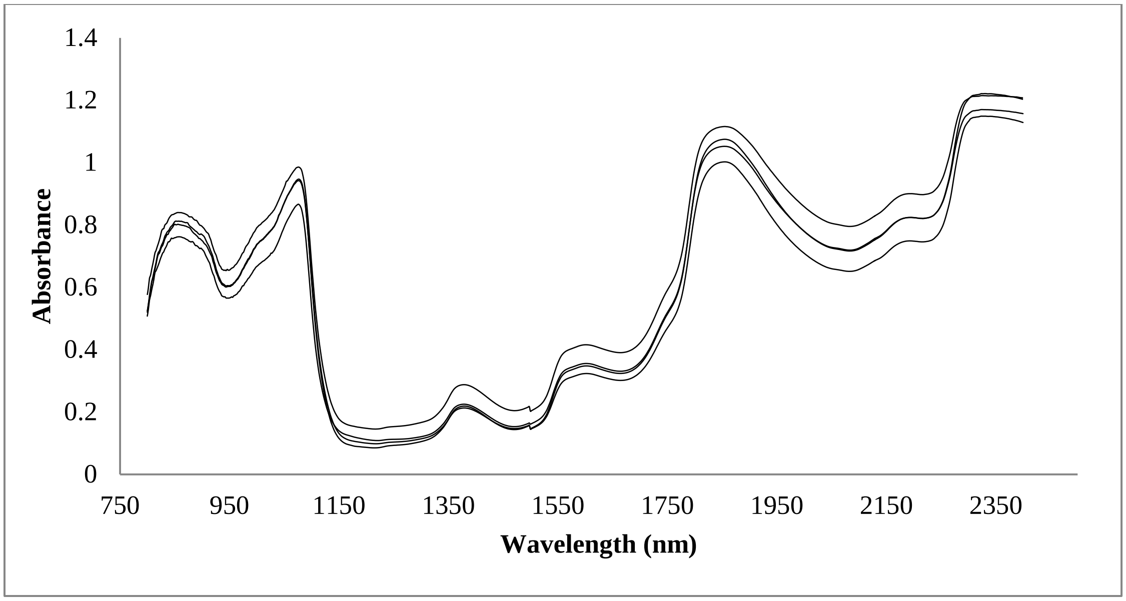

Fig. 5.1: Typical absorbance unprocessed spectra for intact ‘Hass’ avocado fruit.

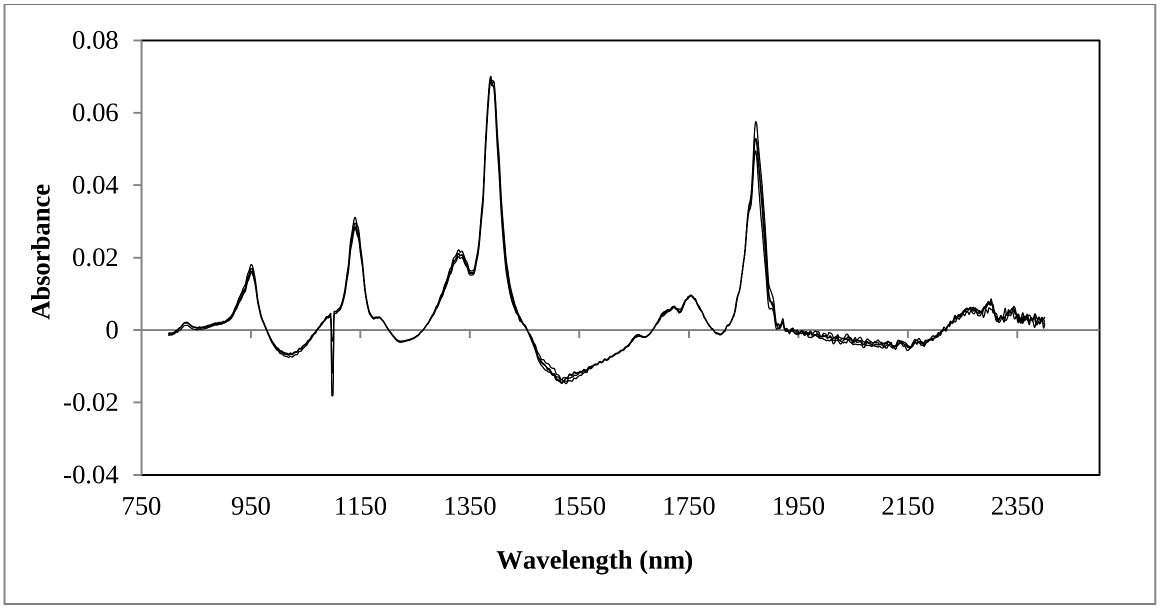

Fig. 5.2: Typical absorbance Standard Normal Variate and Savitzky-Golay first derivative pre-processed spectra intact ‘Hass’ avocado fruit

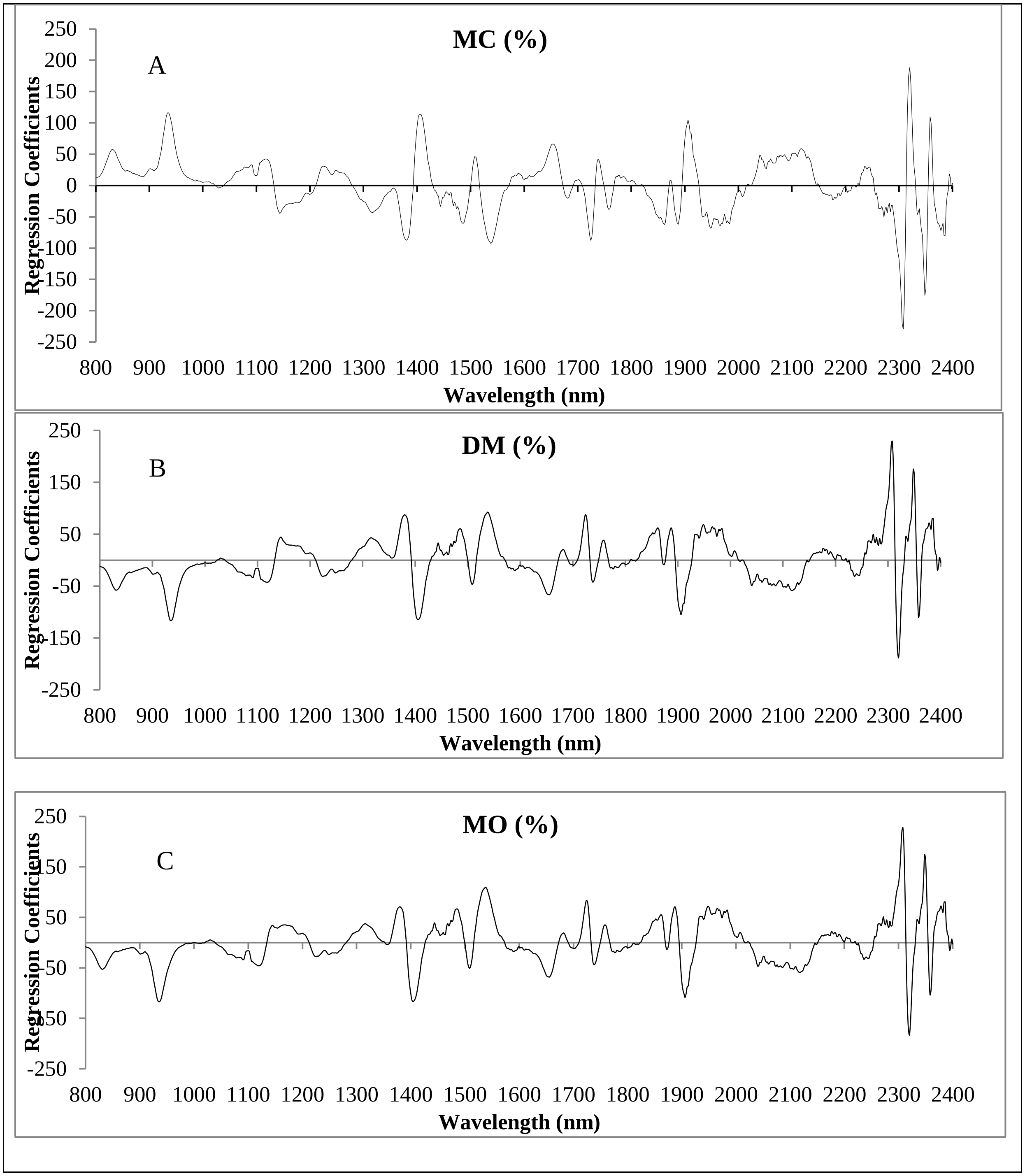

Fig. 5.3: Typical regression coefficients curve of the moisture content, (A) dry matter, (B) and oil content of an intact 'Hass' avocado fruit with 7 PCs and NIR spectra range of 800 – 2400 nm.

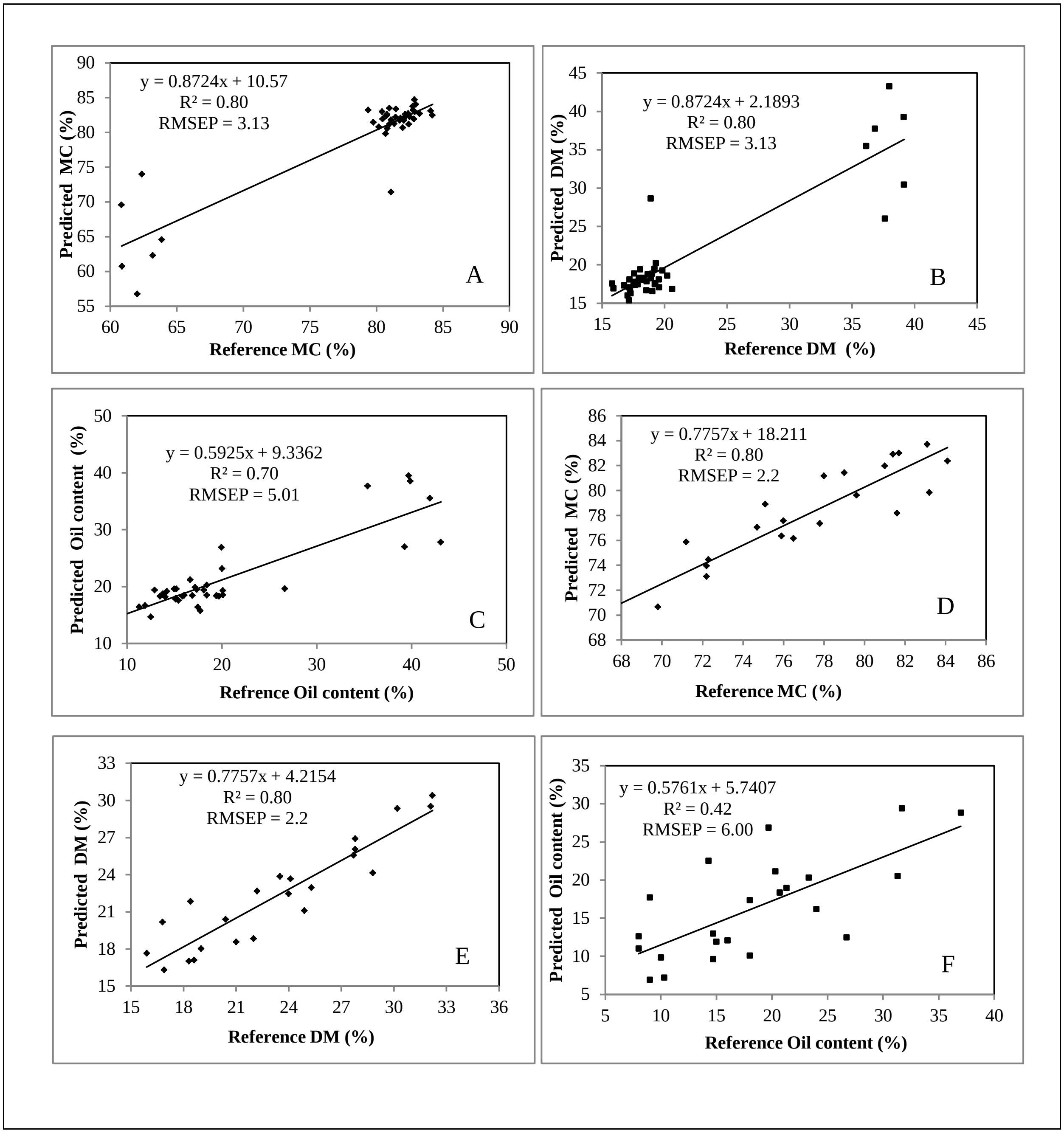

Fig. 5.4: Scatter plots of near infrared predicted against reference constituent values for (A) moisture content 2013 season, (B) dry matter 2013 season, (C) oil content 2013 season (D) moisture content 2014 season, (E) dry matter 2014 season, (F) oil content 2014 season.

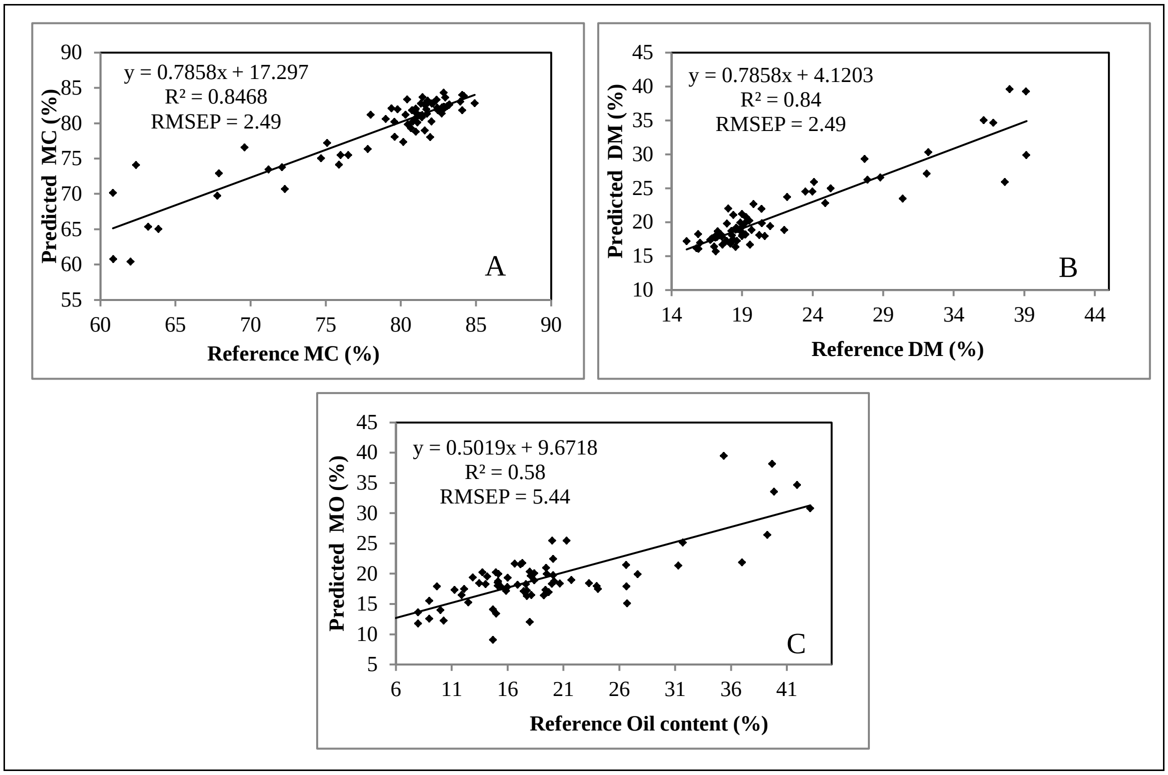

Fig. 5.5: Scatter plots of near infrared predicted against reference constituent values for (A) moisture content 2013-14 seasons, (B) dry matter 2013-14 seasons, (C) oil content 2013-14 seasons.

Table 5. 2: Summary of PLS calibration (cal.) and prediction (pred.) statistics for MC, DM and oil content for intact ‘Hass’ avocado fruit from KwaZulu-Natal, South Africa over 2013 and 2014 seasons.

Parameters	Season	Spectra (RO)	Range	Mean	SD	LV	R^2	RMSEC	RMSEP	SEP	Slope	Bias	Corr.	RPD
MC (%)	Cal. (2013)	100 (5)	58.58-85.58	81.42	4.11	10	0.91	2.27			0.91			
	Pred. (2014)	55 (0)	68.30-81.67	76.27	3.20	10	0.53		3.75	3.78	0.45	-0.15	0.74	0.85
DM (%)	Cal. (2013)	100 (5)	14.42-41.13	18.58	4.11	10	0.91	2.27			0.91			
	Pred. (2014)	55 (0)	18.33-31.67	23.73	3.20	10	0.53		3.75	3.79	0.45	0.15	0.74	0.84
Oil content (%)	Cal. (2013)	100 (5)	10.59-2.14	18.24	4.85	10	0.71	5.16			0.71			
	Pred. (2014)	55 (0)	15.50-30.48	21.09	3.37	10	0.22		7.17	8.51	0.25	2.59	0.60	0.40
MC (%)	Cal.(2013-14)	80 (3)	63.89-85.85	79.69	5.38	7	0.95	1.26			0.95			
	Pred.(2013-14)	72 (0)	60.41-84.32	79.09	5.27	7	0.84		2.49	2.63	0.79	0.45	0.92	2.00
DM (%)	Cal.(2013-14)	80 (3)	14.15-36.11	20.31	5.38	7	0.95	1.26			0.95			
	Pred.(2013-14)	72 (0)	15.68-39.59	20.91	5.27	7	0.84		2.49	2.47	0.79	-0.45	0.92	2.13
Oil content (%)	Cal.(2013-14)	80 (3)	5.58-34.02	18.46	5.21	7	0.67	3.67			0.67			
	Pred.(2013-14)	72 (0)	9.04-39.48	19.41	5.43	7	0.58		5.40	5.44	0.50	0.01	0.77	1.00

RO, Removed outliers; SD, Standard deviation; LV, Latent variable; R^2, coefficient of determination; RMSEC, Root mean square error of calibration; SEP, Standard error of prediction; Corr., Correlation; RPD, residual prediction deviation

CHAPTER 6

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

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1. Introduction

The exact stage of maturity during the growth and development of avocado fruit is difficult to determine because the fruit does not exhibit obvious characteristics that could suggest the optimum state of ‘readiness for harvest’. However, fruit maturity determines the quality of the fruit which invariably affects the fruit’s acceptability of the fruit by the consumers and, hence, the grower’s profitability. Common physical and non-destructive maturity indices of horticultural fruit, which include colour, size and firmness, are not applicable to avocado fruit maturity. Hence, destructive indicators such as mesocarp moisture content (MC), dry matter (DM) and oil content were evaluated in this study. These maturity parameters have individual flaws (Hofman et al., 2013), so that the search for reliable, easily determinable parameter of avocado fruit maturity remains.

2. Percentage mesocarp moisture content (Percentage dry matter)

Generally, the moisture content is regarded as a key component of growth and development of a plant or plant part (fruit). Avocado fruit is composed of between 90% immature fruit to about 60% matured fruit moisture. Its percentage composition at a certain period during fruit growth and development has previously been reported to be associated with harvest maturity (Lee, 1981; Blakey, 2011). Mesocarp moisture content is the parameter currently used by the South African avocado fruit industry as the standard for fruit maturity, while its reciprocal value, DM, is used in other industries such as Australia, Israel, New Zealand, Chile and the

United States (Swarts, 1978; Hofman et al., 2002; Hofman et al., 2013). No observable peak or plateau in MC or DM was exhibited during avocado fruit growth and development that could be linked to the optimum, indicating commercial maturity. In view of this, a further study investigating the relationship between the point at which the seed coat turns brown and dries out as a measure of seed maturity and the corresponding MC is suggested.

3. Percentage mesocarp oil

Avocado fruit is an oleaginous fruit accumulating monounsaturated fatty acids during its development (Ozdemir and Topuz, 2004; Hofman et al., 2013). Over the period of measurement, oil content increased in line with previous reports using similar method of extraction (Meyer and Terry, 2008; Meyer and Terry, 2010)

The results of this study revealed that the MC/DM did not correlate well oil content (Chapter 4). Though this observations has been reported before, the result remain a concern (Blakey, 2011).

4. Fruit growth

The growth of avocado fruit measured in terms of fruit diameter demonstrated similar pattern of growth (Chapter 3). This study revealed that the increase in size of the fruit followed a single sigmoidal growth pattern in line with results reported in literature with respect to the study of avocado fruit growth (Schroeder, 1958; Zilkah and Klein, 1987; Morre-Gordon et al., 1995; Liu et al., 1999; Pak, 2002). However, the significant increase in fruit growth displayed for 'Hass' fruit during the later period of data collection could suggest that avocado fruit follows a double sigmoidal growth curve (McOnie and Wolstenholme, 1982; Pak, 2002). Linear growth phase as observed in this experiment shared close similarities with the morphological studies of fruit growth as reported from previous studies (Cummings and Schroeder, 1942).

5. Total soluble solids (TSS)

Total soluble solids (TSS), measured in °Brix, can be used as a simple, quick and objective indicator of fruit maturity in several horticultural crops (Jha et al., 2006; Jayasena and Cameron, 2008; Watanawan et al., 2014). As avocado fruit maturity has been aligned with its mesocarp sugar (Bertling and Bower, 2006; Blakey, 2011) and, besides oils, the mesocarp contains a large amount of these sugars (Blakey, 2011; Tesfay et al., 2012), the possibility of using TSS indicator as an alternative for measuring avocado fruit maturity was evaluated in this study (Chapter 4). It was revealed, however, that TSS cannot be used to measure maturity of avocado fruit.

6. Non-destructive evaluation of avocado fruit maturity

One objective of this research was to investigate the reliability of near infrared spectroscopy (NIRS) to predict avocado fruit maturity (Chapter 5). Determining the maturity of an avocado fruit is difficult because the fruit does not exhibit obvious characteristics that usually suggest fruit maturity (Stahl, 1933; Lee, 1981; Lee et al., 1983). Previous investigations into avocado fruit maturity have focused mostly on predicting DM (Schmilovitch et al., 2001; Clark et al., 2003; Wedding et al., 2010; Wedding et al., 2011; Wedding et al., 2013), while only a few on assessed MC (Blakey and van Rooyen, 2011), with no investigation was recorded for predicting oil content. This study evaluated the feasibility of FOSS NIRSystem® in a reflectance mode for non-destructive measurements of maturity parameters of avocado fruit.

A predictive model statistics showing the accuracy of prediction developed for MC, DM and oil content has been proposed (Table 5.1 and Table 5.2). NIRS is a non-destructive technique capable of providing non-visible information about comparative proportions of C-H, O-H and N-H bonds in fruit mesocarp (Shenk et al., 2001; Workman and Shenk, 2004; Nicolaï et al., 2007). The prediction results revealed that MC and DM were accurately predicted while the models for predicting oil content were not accurate. The inaccuracy of the model to predict oil content of the avocado fruit was explained to be possibly associated with low concentration of oil in the samples used for the study. The samples had a high moisture percentage which could have possibly masked the concentration of oil in the fruit, consequently limiting the ability of the models to predict the maturity indicator. In view of this, future samples covering a wider distribution from early, mid and late harvesting periods should be included in the calibration model for better prediction results.

The NIRS models obtained in this study also revealed the significance of model robustness in predicting the maturity parameters of future fruit populations. As revealed by Wedding et al. (2013), integrating samples from two seasons into a calibration model improved the prediction ability of the maturity parameters in this study.

7. Conclusion

This study confirmed that MC and DM are still the indicators providing satisfactory measure of avocado fruit maturity. It has been shown that TSS measured in °Brix cannot be used as maturity index of avocado fruit. The study has further demonstrated that FOSS NIRSystem® in reflectance mode can be used a non-destructive instrument to rapidly predict maturity parameters of avocado fruit.

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