

**Ethephon-Induced Changes in Macadamia Nuts: Implications for Dietary Indicators, Nutritional Quality, and Postharvest Shelf Life**

**by**

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## PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Horticultural Sciences, School of Agricultural, Earth and Environmental Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The research was financially supported by the National Research Foundation (NRF), South Africa.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.



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## DECLARATION 1: PLAGIARISM

I, Noluthando Noxolo Hlongwane, declare that:

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(iii) this thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

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## DECLARATION 2: PUBLICATIONS

This thesis involves a compilation of manuscripts and published articles. My role in each manuscript and publication included experimental work, data analysis, and manuscript preparation, and was guided and or supervised by the other authors.

### Chapter 2

1. Aruwajoye, N. N., Mditshwa, A., Magwaza, L. S., Ngidi, M. S. C., & Tesfay, S. Z. Accelerating abscission in macadamia nuts through ethephon: implications for nut quality. *Scientia Horticulturae*, (under review).

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## ABSTRACT

The thriving macadamia nut industry, prized for its nutritional value, grapples with the challenge of inconsistent abscission seasons, leading to variable harvest times and increased operational costs. To address this, the traditional application of ethephon induces uniform nut abscission and streamlines harvesting. However, it is crucial to investigate how ethephon impacts the nutritional quality and postharvest shelf life of macadamia nuts. This study focuses on the effect of ethephon on the postharvest quality of the '788' and 'Beaumont' cultivars, comparing various categories: nuts that successfully abscised due to ethephon (ED), those manually picked from ethephon-treated trees (ET), nuts naturally dropped from control trees (CD), and those manually picked from control trees (CT). Guided by industry-recommended doses, Ethephon 480 SL® was administered using a Cima mist blower to facilitate nut abscission, specifically targeting physiologically mature nuts. The research explores the influence on fatty acids, dietary indicators, biochemical components, and polyphenol oxidase (PPO) activity. Various parameters, including total phenolics, flavonoids, antioxidant activities, sucrose, and total protein, were assessed across treatments using standardized procedures. samples were collected over a 72-day accelerated storage period. The study further assessed the effect of Ethephon on the physical attributes of macadamia during a 56-day accelerated storage, analyzing mass, diameter, colour (L\*, a\*, b\*, and hue angle), and texture. Kernels were roasted at 125 °C for 15 minutes using a hot air oven dryer post drying and dehusking. Models were then developed to predict the firmness and colour parameters of macadamia nuts harvested with ethephon from the '788' and 'Beaumont' cultivars based on drying days and storage conditions. The aim was to determine the minimal processing requirements for achieving satisfactory postharvest nut appearance. Input parameters considered included storage temperature, drying temperature, and storage days,

while the resulting outputs were firmness and colour parameters ( $L^*$ ,  $a^*$ ,  $b^*$ , and hue angle). The range of the input parameters spanned from  $-22^{\circ}\text{C}$  to  $25^{\circ}\text{C}$  for storage temperature,  $35$  to  $57^{\circ}\text{C}$  for drying temperature, and  $0$  to  $70$  days for storage duration. Significant changes were observed in the fatty acid profiles of macadamia nuts exposed to ethephon treatment. Notably, ethephon led to a substantial increase in stearic acid content, reaching  $24,622\ \mu\text{g/g}$  in (ED) after  $72$  days, compared to  $16,764\ \mu\text{g/g}$  in (CD), thereby impacting their nutritional quality. Conversely, ED nuts exhibited a reduction in unsaturated fatty acids (USFAs). Hierarchical clustering analysis identified strong correlations between ED and saturated fatty acids (SFAs) in both ‘Beaumont’ and ‘788’ cultivars, with coefficients of  $0.78$  and  $0.80$ , respectively. This was accompanied by an increase in atherogenic indices, thrombogenic index, and saturation index, along with a decrease in the hypocholesterolemic/hypercholesterolemic ratio. Concurrently, a robust correlation ( $0.97$ ) between sucrose and Polyphenol Oxidase (PPO) was observed in ‘Beaumont’, underscoring the influence of ethylene treatment. Notably, ED ‘Beaumont’ nuts exhibited the highest sucrose content at  $18.63\ \text{mg/g}$ , coupled with elevated PPO activity at  $1.06\ \text{U g-L}$ . For the physical attributes, ED exhibited a notable influence on the ‘Beaumont’ cultivar, particularly affecting parameters such as  $a^*$ ,  $b^*$ , mass, and diameter. Conversely, in the ‘788’ cultivar, Principal Component Analysis (PCA) revealed that CD treatment held prominence, showing elevated values for firmness,  $a^*$ , and  $b^*$ . Furthermore, model development demonstrated significance, with high coefficients of determination ranging between  $0.83$  and  $0.97$ . Notably, under reduced drying days and optimal storage temperature, the models predicted maximum  $L^*$  values of  $76.253$  and  $79.748$  for the ‘788’ and ‘Beaumont’ cultivars, respectively. The firmness model was observed to be significant ( $P < 0.0001$ ) specifically for the ‘788’ cultivar. This study provides a theoretical framework for identifying optimum set points crucial for the effective preservation of both the ‘788’ and ‘Beaumont’ varieties of macadamia nuts, particularly in preventing external discolouration.

The proposed set points aim to enhance the quality and overall market value of these macadamia nut varieties. The overall findings offer a comprehensive perspective on the effects of ethephon application on macadamia nuts. Stakeholders, producers, and processors in the macadamia nut industry can leverage this research to make informed decisions that enhance overall efficiency and quality while ensuring optimal nutritional value and extended shelf life for macadamia nuts.

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## CHAPTER 1: GENERAL INTRODUCTION

### 1.1 Background

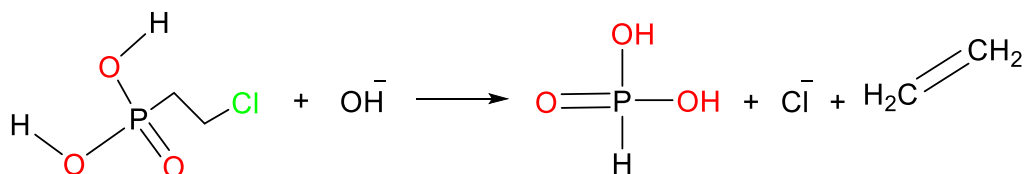
Macadamia trees, originating from the subtropical forests of Australia, are known for their two edible varieties: *Macadamia integrifolia*, recognized for its smooth shell, and *M. tetraphylla*, which is distinguished by its rough shell (Nagao et al., 1992; Niu et al., 2022). Today, macadamia cultivation extends to various regions worldwide, including South Africa, Kenya, the United States, China, southeast Asia, South America, Malawi, and New Zealand (Shuai et al., 2022; Zuza et al., 2023; Nock et al., 2019). In South Africa, commercially significant hybrids of *M. integrifolia*, including both Hawaiian varieties ('246', '344', '660', '741', '788', '791', '800', '814', and '816') and Australian types ('A4', 'A16', and 'Beaumont') are commonly cultivated (Hardner et al., 2009).

Mature macadamia nuts are appreciated for their creamy-white kernels, known for their high nutritional value and abundant oil content (Tan et al., 2020; Navarro and Rodrigues, 2016; Hu et al., 2019). The oil content in *M. integrifolia* is approximately 70.1%, while *M. tetraphylla's* oil content falls within the range of 69% to 78% (Aquino-Bolaños et al., 2017). This oil is rich in monounsaturated fatty acids (MUFA) (constituting 59% of the total fat), vitamin E (tocotrienols and tocopherols), and sterols (Hu et al., 2019; Natasia et al., 2023; Maestri et al., 2020). Macadamia nuts also serve as a source of dietary fiber, vitamins, minerals, and phenolic compounds with antioxidative properties (Wood and Garg, 2011; Minh, 2022). However, they are characterized by a low content of total carbohydrates and protein (Meena et al., 2019; Brufau and Rafecas, 2006). Macadamia nuts are enjoyed in both their raw and roasted states. Roasting not only enhances their flavour but also improves their colour and appearance, providing a crisp texture. (Birch et al., 2010).

## 1.2 Problem statement

The harvesting of mature nuts involves various methods, including manual picking, mechanical shaking, and chemical induction (Gutierrez-Coarite et al., 2018; Trueman, 2002). The process of nut fall occurs gradually over several weeks, with only a small quantity falling each day (Wilkinson, 2005). This gradual process poses challenges associated with manual harvestings, such as labour intensiveness and time consumption (Mason and McConachie, 1994).

Specific cultivars exhibit a distinctive behaviour, retaining nuts in the tree canopy for several months post-maturity (Gama et al., 2020; Trueman, 2002). This phenomenon significantly contributes to prolonged abscission periods and introduces variations in the timing of harvest, even within the same cultivar. The extended retention of nuts in the canopy emerges as a significant factor that influences the dynamics of abscission, thereby impacting the optimal timeframe for harvesting (Trueman, 2002 and Trueman, 2003). Enhancing abscission methods can optimize the nut harvesting process, resulting in improved overall efficiency. For certain cultivars, expediting abscission may involve techniques such as tree shaking or the application of ethephon (Wilkinson, 2005). Ethephon, a plant growth regulator releasing ethylene, is a long-established method to stimulate various plant developmental processes, including the shedding of flowers and nuts.



**Figure 1.1** Illustrating Ethephon Breakdown - chemical Structure and Release Mechanism.

When applied to tree nuts, ethylene ensures uniform abscission, facilitating a more efficient and synchronized harvesting process. A study by Trueman (2002) demonstrates that

the use of ethephon, mechanical tree shaking, or a combination of these methods effectively speeds up crop removal from the tree.

The duration of nut retention on macadamia trees introduces significant disparities in quality among cultivars. These disparities may manifest in various aspects such as kernel oil and nutritional composition (Kazantzis et al., 2003). Understanding these differences is crucial for the macadamia nut industry, as they can impact market value and consumer preferences. In a pivotal study conducted by Gama et al. (2020), the researchers explored the nuances of nut quality concerning extended periods of retention on the tree. The study illuminated substantial distinctions in quality when compared to nuts harvested at earlier stages, where late-harvested nuts had a shorter shelf life and lower oil quality compared to those harvested earlier (Gama et al., 2020). To aid harvesting and promote abscission, ethylene-releasing agents, such as ethephon, are used. Trials conducted in South Africa reported a significant 70-80% reduction in nut retention on the ‘Beaumont’ cultivar when ethylene-releasing agents were used (Penter et al., 2003). There is a specific need to delve into the contrast between nuts that undergo successful abscission due to ethylene-releasing agents, such as ethephon, and those that persist on the tree.

#### **1.4 Research motivation**

The utilization of ethephon to trigger nut abscission has demonstrated its effectiveness in mitigating harvesting expenses, establishing a uniform harvesting window, and facilitating efficient orchard management (Trueman et al., 2002). However, it is essential to acknowledge the limited availability of research reports that comprehensively explore the influence of ethephon on nut quality across various cultivars during the postharvest storage period. Furthermore, it is noteworthy that ethylene, a key component in ethephon, is a known

promoter of senescence and fruit ripening (Lee et al., 2021; Iqbal et al., 2017). The ripening process is a unique and plant-specific developmental phase that leads to a significant enhancement in sensory attributes, including aroma, flavour, texture, and nutritional composition (Pech et al., 2012). This natural phenomenon highlights the importance of understanding the role of compounds like ethephon in modulating these attributes.

In a study conducted by Wang et al. (2016), the application of ethephon at a concentration of 500 mg/L on fresh in-husk walnuts demonstrated its effectiveness in inhibiting the activities of polyphenol oxidase (PPO), leading to a noticeable reduction in the browning of green husks. Additionally, this treatment resulted in a significant increase in the total phenolic (TP) content in green husks of walnuts (Wang et al., 2016). Li et al. (2004) also observed that the application of ethephon at a concentration of 400 mg/L led to an increase in polyphenol oxidase (PPO) levels in the leaves compared to the control.

Furthermore, Zhang et al. (2012) reported that the application of ethephon on kiwifruit resulted in increased soluble solid concentrations and total phenol content. In a similar study by Reis et al. (2020), ethephon's influence on strawberries was complex, affecting various fruit attributes, including mass, diameter, length, firmness, anthocyanins, amino acids, and volatiles. However, the magnitude of these effects was contingent on the fruit's specific developmental stage (Reis et al., 2020). Moreover, the impact of ethephon can vary significantly based on the timing of application and cultivars, likely influenced by inherent variations in phenology and hormonal ratios (Nunn et al., 2020).

Thus, understanding the interplay of ethylene and the ripening process is crucial in evaluating the overall impact of ethephon on nut quality and its potential implications in horticultural practices. Additionally, evaluating the impact of harvesting macadamia nuts using ethephon is essential because proper postharvest procedures play a crucial role in preventing both physical and chemical damage. Such damage can significantly compromise

the overall quality of the nuts. Macadamia nut processing includes postharvest stages such as harvesting, de-husking, drying, cracking, grading, and packaging (Srichamnong and Srzednicki, 2015). Understanding the effects of ethephon during the harvesting stage is particularly important to optimize the subsequent processing steps and ensure the preservation of high-quality macadamia nuts.

### **1.5 Aim and objectives**

This study aims to investigate the effects of ethephon on the postharvest quality of two macadamia nuts cultivars namely: ‘788’ and ‘Beaumont’.

The following specific objectives were undertaken in order to achieve the above-mentioned aim:

- i. examine how ethephon treatment affects individual fatty acids and dietary indicators in macadamia nuts that abscised due to the treatment as well as those that naturally abscised during postharvest storage.
- ii. evaluate the variation in the biochemical composition and polyphenol oxidase activity between the nuts harvested directly from the tree and those collected from the orchard floor.
- iii. evaluate the effect of preharvest ethephon application on the physical attributes of roasted macadamia nuts.
- iv. investigate the singular and interactive effects of storage temperature, drying days, and storage period on the firmness and colour attributes of macadamia nuts harvested with ethephon (‘788’ and ‘Beaumont’ cultivars) using Response Surface Methodology (RSM).
- v. optimize firmness and colour-related output parameters ( $L^*$ ,  $a^*$ ,  $b^*$ , and Hue angle) for both cultivars, considering the comprehensive influence of all input parameters, to attain the most favourable values.

## 1.6 Thesis outline

This thesis is structured into six chapters following the research paper format.

**Chapter 1** provides an overview of the study's context, including its background, problem statement, and the underlying motivation for the research.

**Chapter 2** is a comprehensive review of relevant literature, with a specific focus on macadamia production and cultivation. It examines the application of ethephon in the macadamia nut industry, its role in accelerating nut abscission, and its potential impact on nut quality.

**Chapter 3** highlights the influence of ethephon application on the fatty acid profiles and dietary indicators of two macadamia cultivars, namely '788' and 'Beaumont', during the postharvest storage period.

**Chapter 4** explores the effects of ethephon application on the biochemical parameters of two raw macadamia cultivars, '788' and 'Beaumont', during the postharvest storage period.

**Chapter 5** examines how ethephon application influences the physical attributes of roasted macadamia cultivars '788' and 'Beaumont'.

**Chapter 6** employs Response Surface Methodology (RSM) to characterize the individual and combined impacts of storage temperature, drying days, and storage period on the firmness and colour attributes of macadamia nuts harvested with ethephon ('788' and 'Beaumont' cultivars). Additionally, the chapter focuses on optimizing the colour-related output parameters ( $L^*$ ,  $a^*$ ,  $b^*$ , and Hue angle) for both cultivars, ensuring a holistic consideration of all input parameters to achieve the most favourable values.

**Chapter 7** presents a summary of the research findings, draws overall conclusions, and provides insights into potential avenues for future research. It also offers practical recommendations based on the study's outcomes.

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## **CHAPTER 2: ACCELERATING ABSCISSION IN MACADAMIA NUTS THROUGH ETHEPHON: IMPLICATIONS FOR NUT QUALITY A REVIEW**

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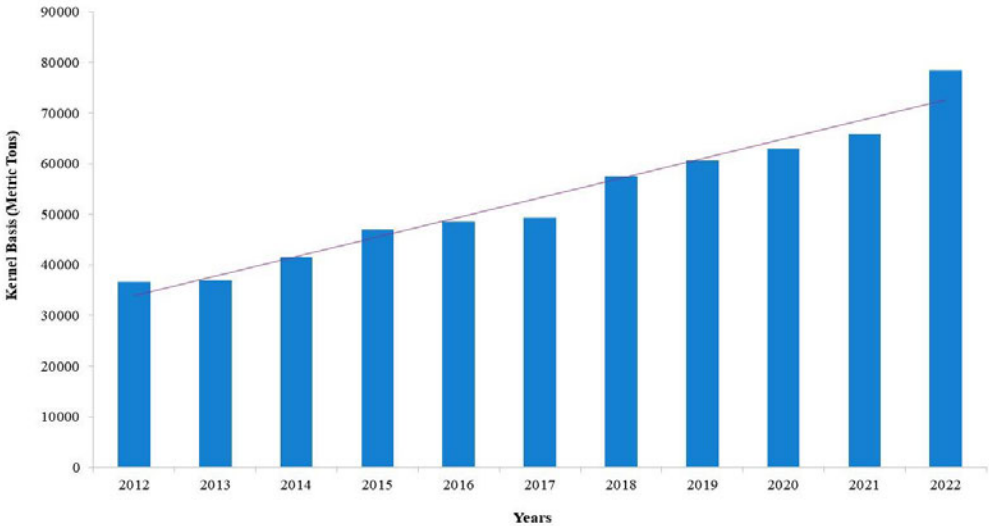
## 2.1 Abstract

Macadamia nut is a valuable crop due to its high nutritional value characterized by a notable content of healthy monounsaturated fats, dietary fiber, antioxidants, and essential minerals like magnesium and potassium. As a result, it has a constantly growing global production from countries such as Australia, South Africa, China, and the USA. However, macadamia harvesting is challenged by variations in abscission seasons which result in different harvest times (early and late). Meanwhile, delayed harvesting has been linked to the reduced kernel quality of macadamia nuts. To mitigate this challenge, ethephon has been used to accelerate abscission which subsequently prevents delayed harvesting. This literature review aims to give a brief overview of the use of ethephon, its mechanism, and possible side effects on macadamia nut quality. Findings reveal that the major side effects of the application of ethephon for macadamia harvesting are associated with ethylene usually released from ethephon during the process of acceleration of plant cell senescence and this has implications on nut quality and shelf. Thus, this review bridges the information gap between the detailed mechanisms involved in the application of ethephon for early macadamia harvesting and its side effects on the macadamia nut quality. This information can assist farmers, stakeholders, and entrepreneurs by influencing major decisions for profitable macadamia nut production.

**Keywords:** *Ethephon, ethylene, macadamia nuts, abscission, kernel quality*

## 2.2 Introduction

Macadamia nut is a high-value crop that is cultivated in many countries, including Australia, South Africa, Kenya, the USA, China, Guatemala, Malawi, Zimbabwe, and Brazil (Bandason et al., 2022). Over the past decade, global macadamia production has exhibited steady growth, surpassing 70,000 tons in the year 2022, as depicted in Figure 2.1 (International Nut and Dried Fruit Council, 2022). Moreover, in 2022, the industry witnessed a significant growth in its annual revenue, surpassing 300 million USD compared to 7.3 million USD in 1996 (Bouarakia et al., 2023). Leading the global market, South Africa and Australia accounted for 31% and 23% of exports, respectively (International Nut and Dried Fruit Council, 2022).



**Figure 2.1** Global Macadamia production in Metric tons (kernel basis) between 2012 and 2022. Data extracted from the International Nut and Dried Fruit Council, (2022).

Macadamia nut is native to Australia where there are about four species of which only two produce edible nuts which are *Macadamia integrifolia*, known as the smooth shell, and *Macadamia tetraphylla*, referred to as the rough shell (Dailey et al., 2015; Nagao et al., 1992). These species are part of the Proteaceae botanical family (Canneddu et al., 2016). Notably,

these nuts stand out for their rich content of beneficial monounsaturated fats, dietary fiber (6g/100g), protein (9.2%), total sugar (4.22%–4.75%), antioxidants, as well as essential minerals like magnesium and potassium (Schlörmann, 2017; Buthelezi et al., 2019; Yang et al., 2009; Rahman et al., 2021). Though native to Australia, commercial production of Macadamia was initiated in Hawaii during the mid-1920s (O'Connor et al., 2019) and is now grown with its hybrid across the world. In Australia, the harvest of macadamia nuts typically takes place from March to September (Brinkhoff et al., 2021) whereas in South Africa, growers undertake the harvest and processing of macadamia nuts from February to August (SAMAC, 2021). Major producers such as Australia and South Africa export up to 99% of their yield to countries such as the USA, China, Japan, and Vietnam (Shabalala et al., 2022). Prior to exports, macadamia fruits are usually harvested by collecting nuts from the orchard floor following natural fruit abscission (Walton and Wallace, 2009). Harvesting time varies due to several reasons such as cultivar type, rapid fruit abscission, lack of harvesting equipment, inadequate on-farm storage, the inability of processors to receive the product, and weather conditions (Trueman et al., 2002; Walton and Wallace, 2009). Extended abscission seasons have been identified as a significant factor contributing to the delayed nut drop of certain cultivars (Trueman et al., 2002). Consequently, nuts remain on trees for a longer duration, adversely affecting the quality of the kernels. Macadamia, being a tree nut known for its high oil content and fatty acids, is particularly susceptible to oxidative rancidity and rapid deterioration (Gama et al., 2018). Walton and Wallace, (2009) reported that when the harvest is delayed, the rate of kernel deterioration may exhibit a higher pace in later-harvested nuts when compared to those harvested earlier. Kernels from late-harvested nuts have higher peroxide values which indicate that lipid oxidation is initiated several months before harvest whilst nuts are still attached to the tree (Gama et al., 2020).

Therefore, it is crucial to harvest nuts soon after maturity is recommended to reduce quality losses related to delayed harvest (Walton and Wallace, 2009; Gama et al., 2020). However, variation in abscission periods necessitates the introduction of cultural practices that will hasten nut abscission. This can be accomplished by mechanically shaking the trees or by applying ethephon (Quinlan et al., 2008). Mechanical shaking of trees has been reported as an effective method of promoting nut abscission (Quinlan et al., 2008). However, the combined use of ethephon and mechanical shaking of the tree has been proven to be more efficient (Trueman et al., 2002). Ethephon is applied first before shaking the tree to reduce fruit removal force but lone usage of ethephon is the most practical (Walton and Wallace, 2008). Ethephon, is an ethylene-releasing compound that plays accelerates abscission in mature nuts by facilitating their detachment from the tree (Trueman, 2010). Thus, challenges arising from delayed abscission and multiple harvest periods can be mitigated through the use of ethephon.

Therefore, the study aims to review current knowledge regarding the effect of ethephon on the harvesting of macadamia nuts. Moreover, it explores possible side effects of ethephon on kernel quality and shelf life. The findings of this review will contribute to knowledge that can inform major decisions for profitable macadamia nut production.

### **2.3 Macadamia nut developmental stage, maturity, and senescence**

The developmental stages of macadamia nuts, encompassing maturity and senescence, intricately align with the phenological cycle of the macadamia tree. This cycle entails a series of stages, including floral initiation, flowering, nut set, nut development, oil accumulation, and the drop of mature nuts (Stephenson et al., 2003). Significantly, mature macadamia trees exhibit a capacity to produce over 10,000 inflorescence racemes, each of which bears about 100 to 300 flowers, serving as the catalyst for the subsequent flowering process (Prasannath et

al., 2021). This means each tree can have close to 1 to 3 million flowers. However, the number of flowers is not proportional to the number of harvestable nuts. According to Howlett et al. (2015), only about 0,3% of the flowers result in harvestable nuts. This is because, after anthesis, a great percentage of flowers drop because of being unfertilized (du Queens et al., 2018). Macadamia is partially self-incompatible, and bee pollinated. Certain macadamia cultivars have proven self-compatibility, however, even in the case of such cultivars cross-pollination remains majorly important for yield improvement. Cross-pollination has been shown to increase final fruit yield by up to 95% compared to self-pollination (Trueman, 2013; da Silva Santos et al., 2020). However, pollination could be limited in regions that practice non-pollinator-friendly management and high pesticide application (Grass et al., 2018). Once the fruit set has been initiated, nut growth increases until it reaches mature size and hardened shell (Nortjé, 2018). Subsequently, kernel sugars are converted to oil (Nortjé, 2018). Kernels with high sugar content are characterized as immature (Gama et al., 2018; Wall and Gentry, 2007). The highest quality kernels are described by an oil accumulation of 72 -78 % (Wall, 2013). Following oil accumulation, the natural process of mature nut drop occurs in macadamia trees. However, this critical stage exhibits variation among cultivars and harvesting seasons, as highlighted by Trueman (2013). The extended nut drop timing poses a significant challenge in the macadamia nut industry. In the next section, we will explore the necessity and potential benefits of utilizing plant growth regulators, such as ethephon, to address the challenges associated with nut drop variation.

#### **2.4 Necessity for the use of ethephon and other plant growth regulators**

Different plant parts encounter several challenges, including issues like fragile stalks that can lead to lodging, variations in maturity levels causing uneven ripening and diminished biochemical and physicochemical quality, irregular abscission, and decreased precocity

(Duyvelshoff and Cline, 2013; Ferrara et al., 2016; Ahmed et al., 2019; Zhang et al., 2019; Gao et al., 2020; Maduwanthi et al., 2021). Plant hormones such as ethylene, abscisic acid, auxins (Duyvelshoff and Cline, 2013), cytokinins, gibberellins, salicylic acid, and jasmonates or agrochemicals containing such compounds as active ingredients are used to achieve desired quality attributes (Ferrara et al., 2016; Wang et al., 2018). Several reports have alluded to the positive effects of cytokinins on branching, delay of senescence, nutrient metabolism, and flower, and seed set control (Koprna et al., 2016). Similarly, auxins have been reported to successfully inhibit premature ripening with eventual fruit quality improvement (Dal Santo et al., 2020). Recently, the premature ripening of grapes prompted by the rising global temperature (climate change) was mitigated with the use of auxins (Böttcher et al., 2021). Dal Santo et al. (2020) suggests that delaying cell expansion could be the possible mechanism of auxin to delay fruit ripening. The regulation of fruit ripening can be achieved through the interaction of plant hormones. Studies have demonstrated that there is an interaction between auxin and ethylene signalling to regulate fruit ripening (Hao et al., 2015; Zhang et al., 2019). Ethylene promotes ripening, and its biosynthesis is regulated during fruit ripening (Liu et al., 2021). Moreover, ethylene is involved in seed germination, flowering, organ senescence, cell death, and response to abiotic stresses and pathogen attacks (Gao et al., 2020). The important function of plant hormones on plant growth and development necessitates their use as agrochemicals (Koprna et al., 2016). Ethephon is among the many agrochemicals that have been widely explored in agricultural practices.

#### ***2.4.1 The use of ethephon in commodities and agricultural practices***

Ethephon is mixed with water to form a spray solution, which is then applied by spraying on crops, including macadamia nut trees (Walters et al., 2018). Once applied, ethephon penetrates through the stomata and cuticles to the apoplast pathway (Bhadoria et al.,

2018). After application, ethephon then decomposes to form ethylene, chloride, and phosphate (Bhadoria et al., 2018; Kundu et al., 2019). Ethylene released from ethephon regulates many aspects of fruit development and has been reported to have notable effects on ripening, ripening-related genes, abscission, stalk strength, physicochemical properties, and flowering (Duyvelshoff and Cline, 2013; Ferrara et al., 2016; Ampa et al., 2017; Zhang et al., 2019; Maduwanthi et al., 2021). The application of ethephon on bananas resulted in reduced firmness which is an indication of the start of fruit ripening (Maduwanthi et al., 2021). A similar trend in firmness was observed in Mango and kiwi fruit (Ampa et al., 2016; Jawandha et al., 2016). The presence of ethylene enhances the conversion of starch to sugars and the reduction of organic acid (Ampa et al., 2016). Additionally, it has been reported to suppress stem elongation, increase stem diameter, and reduce apical dominance (Walters et al., 2018). Ethylene is also known as a feminizing hormone in plants and its application has the potential to induce partial sexual change in dioecious plants (Moon et al., 2020). In recent studies, ethephon has been used to increase the yield of *Cucurbitaceae* plants that produce female, male, and bisexual flowers. Ethephon was used specifically to enhance female flower formation (Li et al., 2021). Furthermore, ethephon induced morphological alteration by decreasing plant height, thickened epicotyls, and inhibiting root elongation (Li et al., 2021). Similarly, ethephon improved the stalk mechanical strength of the maize plant by altering internode morphological traits (Zhang et al., 2019). The efficacy of ethephon in altering morphological traits suggests that it could also be beneficial for agricultural cultivation activities such as dwarfing the stem for seedling strengthening (Li et al., 2021). The application of ethephon has been reported to increase tea leaf yield by inhibiting blooming (Zhang et al., 2022). This redirection of the plant's nutrients and water towards leaf production contributes to a higher yield of tea leaves. Ethephon is also utilized as a cost-effective chemical thinner, serving as an alternative to the labour-intensive process of manual

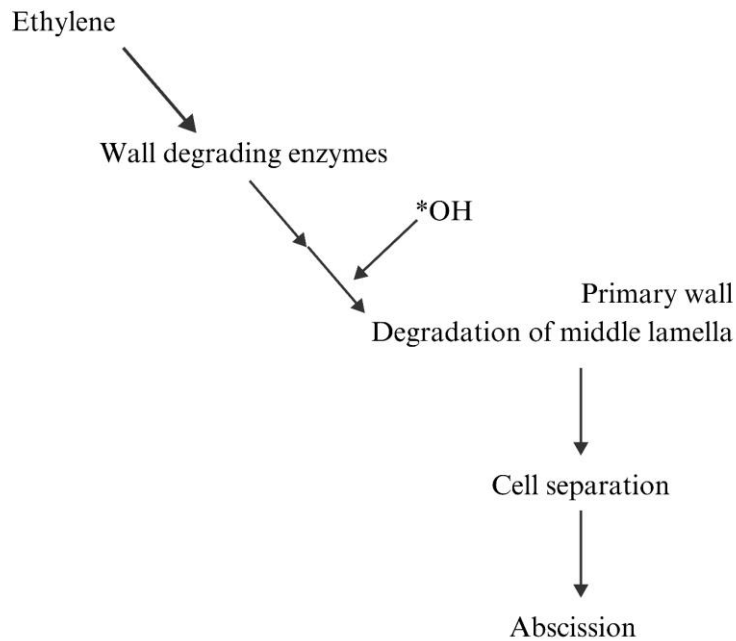
thinning (Ali et al., 2012). Fruit thinning involves the removal of excess fruitlets to enhance fruit size, colour, and quality at harvest (Yuan, 2007). Yuan, (2007) indicated that applying Ethephon at a concentration of  $400 \mu\text{LL}^{-1}$  effectively thinned apples. Moreover, Sarkadi et al. (2012) demonstrated that applying 350-500 ppm ethephon to thin peaches resulted in improved fruit colour.

#### ***2.4.2 Nut abscission properties of ethephon***

Abscission is a developmental process that occurs naturally in plants resulting in the shedding or separation of leaves, flowers, fruit, and branches from the parent plant (Osborne and Morgan, 1989; Meir et al., 2019). The process takes place at the abscission zone and can be divided into four phases. The first step is described by the differentiation of cells that are present within the abscission zone. This is followed by the second step whereby these cells are expected to acquire the competence to respond to abscission signals. Once this is achieved, the third step proceeds which is the loosening of the cell wall and cell separation (In this case: nut drop). Finally, a protective layer is formed over the organs remaining on the parent tree (Kim, 2014; Patharkar et al., 2018; Liu et al., 2020b; Rai et al., 2021). Two models of abscission zone activation have been suggested: senescence-triggered abscission and correlatively driven abscission (Bangerth, 2000; Giulia et al., 2013; Ackerman and Samach, 2015). Senescence-triggered abscission is a natural process that occurs in ripe fruits and leaves, where the shedding of these parts is developmentally programmed (Giulia et al., 2013). In this model, as the leaf or fruit matures ethylene production is triggered in turn reducing auxin production and basipolar transport of Indolyl acetic acid to the abscission zone. This leads to increased sensitivity of the tissue to ethylene, ultimately initiating abscission through enzymatic processes and cell separation (Bangerth, 2000). However, this model specifically focuses on mature fruits and doesn't account for the abscission of young

developing fruits. On the other hand, Correlative driven abscission involves the natural shedding of young developing fruits, driven by the interrelation between neighbouring fruits and nearby shoots (Giulia et al., 2013). This process reflects a plant's ability to regulate its fruit load based on nutritional factors, ensuring effective resource utilization (Giulia et al., 2013). Consistently, a fruitlet with a stronger ability to attract and utilize nutrients is more likely to accumulate greater resources compared to a neighbouring fruitlet with a lower capacity. As a result, the fruitlet possessing higher "sink strength" has a better chance of survival (Ackerman and Samach, 2015). Furthermore, the movement of a plant hormone known as auxin within fruitlets, encounters specific points termed "autoinhibitory junctions" along the stem, or peduncle (Larson et al., 2022). These junctions act as points of interaction where auxin flow from dominant fruitlets can hinder the movement of auxin from less dominant ones. The reduced downward movement of auxin through the pedicel, along with heightened sensitivity to ethylene in the pedicel's abscission zone (AZ), triggers the abscission of fruitlets (Hagemann et al., 2015). Although ethylene does contribute to the final stages of fruit detachment, it is not the initial catalyst for the process, as is the case with abscission prompted by senescence (Bangerth, 2000). The ability of ethephon to release ethylene has made its use practical in accelerating the abscission of macadamia nuts. Ethylene released from ethephon causes the cell inside the pedicel abscission zone to produce cell wall degrading enzymes that are responsible for breaking down the pedicel abscission zone which leads to the detachment of leaves, flowers, fruit, and branches from the parent plant (Hagemann et al., 2015). A systematic process of ethylene-induced abscission is illustrated in Figure 2.2. Briefly, when ethylene is released, wall-degrading enzymes (cellulose and polygalacturonase included) are synthesized and thereafter dissolve the primary cell wall and the middle lamella. This step is followed by the breakdown of the cell wall which results in cell separation and organ shedding (Yamada et al., 2015).

## Ethylene-induced Abscission



**Figure 2.2** Illustration of ethylene-induced abscission process. Adapted from (Yamada *et al.*, 2015).

### ***2.4.3 Evaluation of the application of ethephon for macadamia nut abscission***

The use of ethephon to induce abscission in macadamia nuts dates back as early as the 1980s (Kadman *et al.*, 1983; Gallagher *et al.*, 1985; Stephenson *et al.*, 1987). The high costs associated with multiple harvest times and cultivars exhibiting late-season abscission are the major motivations for the use of ethephon to promote nut abscission. Reports investigating abscission without the use of ethephon revealed that fruit maturation and the timing of abscission are independent processes in macadamia nuts (Trueman *et al.*, 2000). This necessitates the use of ethephon on cultivars exhibiting delayed abscission. Ethephon applied

on 'A16' (a macadamia cultivar exhibiting late-season abscission) effectively induced abscission and had no adverse effects on nut quality but showed negative effects on yield (Trueman, 2003). Earlier studies conducted by Richardson and Dawson (1993) on the use of ethephon to induce abscission in macadamia trees observed a reduction in yield in the following season when ethephon was applied either 2 or 4 weeks after nut maturity (Richardson and Dawson, 1993). This yield decline is primarily attributed to the significant loss of leaves caused by the application of ethephon (Trueman, 2003). Philips (1970) had previously reported that a decrease in yield is associated with leaf loss. Furthermore, Burns et al. (2003) also found that ethephon application led to leaf drop just two days after its application, providing further evidence of its negative impact on foliage. Photosynthesis plays a crucial role in determining crop yield (Wu et al., 2019). It relies on various factors, including the proportion of biomass allocated to leaves during vegetative growth, rates of leaf growth, leaf size, and leaf longevity (Long et al., 2015). These factors collectively influence the efficiency of light interception and photosynthesis. When ethephon is applied to plants, it induces leaf loss, resulting in a reduction in the overall number of leaves available for photosynthesis. Consequently, the plant's capacity to assimilate carbon dioxide (CO<sub>2</sub>) through photosynthesis is diminished in turn resulting in a decline in yield (Trueman, 2003). However, a recent study by Liu et al. (2020b) demonstrated that leaf loss does not always lead to yield loss (Liu et al., 2020b). In fact, optimized leaf removal can enhance light distribution, subsequently increasing photosynthetic capacity and the post-silking source-sink ratio (Liu et al., 2020b). As a result, this improvement can positively impact crop yield.

Nevertheless, the use of ethephon remains practical and beneficial to prevent delayed harvesting periods. Ethephon has also been used in conjunction with mechanical shakers (Trueman et al., 2002). Over the years, research has progressed into achieving more efficient

ways of enhancing the use of ethephon for nut abscission such as attaining appropriate pH of the ethephon solution as well as effective concentrations (Jing et al., 2018). Ethephon is a strong acid that can alter the final pH of the solution, consequently affecting its effectiveness (Camberato et al., 2014). Higher ethephon application efficiency may also be attained if the nut age is put into consideration during application since research suggests that nut age influences differential response to ethephon application (Nagao and Sakai, 1988). The sensitivity to ethephon treatment varies depending on the stage of nut development after flowering. Nuts that are small, which are around 3–7 weeks after flowering, and full-size nuts nearing maturity, which are approximately 28–30 weeks after flowering, are responsive to ethephon (Jones, 2002). On the other hand, midsize nuts do not show significant sensitivity to the treatment (Jones, 2002). This suggests that timing the ethephon application correctly can maximize the efficiency of abscission induction, ultimately enhancing nut drop.

#### ***2.4.4 Factors influencing the efficacy of ethephon***

Ethephon is a compound that releases ethylene, with its stability being pH-dependent. For instance, in acidic solutions, ethephon remains stable, whereas it undergoes hydrolysis in plant tissues with higher pH levels (Torres and Asín, 2023). This hydrolysis results in the release of ethylene, which, upon external application, triggers ethylene-dependent processes such as flower or fruit abscission in plants (Torres and Asín, 2023). In flowers and climacteric fruit, ethephon is exogenously applied as a treatment but induces endogenous ethylene biosynthesis. Two distinct systems have been acknowledged in regulating ethylene production. The first is System 1, where ethylene suppresses its biosynthesis during vegetative growth. On the other hand, System 2 operates exclusively during climacteric fruit ripening in specific species, characterized by an autocatalytic ethylene biosynthesis process that intensifies production (Argueso et al., 2007). Autocatalytic ethylene production involves

ethylene-induced upregulation of enzymes such as 1-aminocyclopropane-1-carboxylate synthase and 1-aminocyclopropane-1-carboxylate oxidase, which play a crucial role in the rate-limiting reaction of ethylene biosynthesis (Gorny and Kader, 1997). Marei and Crane, (1971) reported that application of ethylene autocatalytically stimulates the tissue to produce ethylene, consequently reaching a physiologically active concentration that promotes growth and accelerates ripening. However, a recent study by Reig et al. (2016) on loquat fruit, suggests that the ethylene released by ethephon might have a transient effect, lacking the ability to trigger the autocatalytic biosynthesis of ethylene and, as a result, the subsequent ripening process (Reig et al. 2016). The use of ethephon has been associated with inconsistency due to efficacy-dependent factors such as cultivar, climate, chemical rate, time of application, and spray additives (Torres et al., 2021). Some cultivars exhibit a differential response to ethephon. For example, the Kiwi fruit has two commercially important species which are *Actinidia deliciosa* and *Actinidia chinensis*. The *A. chinensis* has been reported to have a higher endogenous ethylene production. When ethephon is applied to such species, the species is likely to have an enhanced ethylene biosynthesis due to its higher endogenous ethylene production compared to *A. deliciosa* (Ampa et al., 2016; Torres et al., 2021). Temperature also has a significant role in ethephon degradation. At high temperatures, ethephon degradation and subsequent release of ethylene are rapid which results in a reduced half-life of ethephon (Ali et al., 2012; Walters et al., 2018). Application of ethephon under low temperatures has been associated with poor abscission whereas, in high temperatures, ethephon application may cause over-thinning or massive defoliation (Yuan, 2007). Concerning application rate and time, early application with high concentration has been reported to be effective (Li et al., 2019). However, since ethephon achieves various processes that tend towards senescence, its use needs to be well understood about the fruit or flowers on which it is applied to mitigate the inconsistencies associated with the use of ethephon.

#### ***2.4.5 Application of ethephon on nut trees***

Ethephon has many beneficial effects, however, many undesirable side effects have been reported as shown in Table 1 (Atli et al., 2021; Grijalva-Contreras et al., 2011; Wang et al., 2016; Kamiab et al., 2020). Ethephon is known to be an effective defoliant inducing synchronized flowering with a high defoliation rate of about 96.53% (Jhade et al., 2019). However, the loss of young and photosynthetic leaves is harmful to the plant as opposed to the older and shaded ones which allow for better light interception within the canopy (Sanderson et al., 1997). Ethephon has also been used to achieve fruit abscission or loosening but has been achieved alongside unwanted defoliation on some rare occasions (Burns et al., 2008). For example, ethephon applied on table olives to achieve fruit abscission successfully reduced fruit detachment force, however, resulted in leaf defoliation (Burns et al., 2008). Moreover, when ethephon was applied to increase maize yield, it resulted in a decreased leaf area index and pronounced leaf senescence which in turn decreased yields (Khalil et al., 2016; Li et al., 2019). Khalil et al. (2016) reported that high dose ethephon application caused leaf defoliation and nut removal with a subsequent reduction in the total kernel weight of pecan nuts (Khalil et al., 2016). Similarly, the use of ethephon reduced the number of pistachios nuts per ounce. This implies a reduction in the market value of nuts as this is directly related to the obtainable number of nuts per ounce (Rahemi et al., 2007). Ethephon application-maintained kernel quality when walnuts (cultivars Serr and Lara) were harvested within 10 and 14 days of treatment (Lang et al., 2010). This suggests that delaying the harvest of these walnut cultivars beyond this period could significantly affect kernel quality. In some cases, the side effects from the use of ethephon pose no detrimental effects as shown in Table 2.1 (Lang et al., 2010). Ethephon has been previously studied for its potential application on almond trees, as almond bud flowers are vulnerable to damage at freezing temperatures (Omidifard and

Gharaghani, 2022). In their study, (Grijalva-Contreras et al., 2011) assessed the effect of ethephon on delaying bloom and found that application of ethephon at the 10% leaf drop stage resulted in a delay of bloom by 7, 8, and 9 days at concentrations of 75, 150, and 300 mg L<sup>-1</sup>, respectively. Although available literature on the effect of ethephon on the internal kernel quality of tree nuts is limited, studies reporting the subject on selected nuts are presented in Table 2.1.

**Table 2.1** Effect of ethephon on selected tree nuts

<b>Tree nut</b>	<b>Concentration</b>	<b>Benefit</b>	<b>Side-effect</b>	<b>References</b>
Almonds ( <i>Prunus dulcis</i> )	75–300 ppm	Mediated bloom delay	Caused up to 3-fold yield reduction	(Grijalva-Contreras et al., 2011)
Pecan ( <i>Carya illinoensis</i> )	200, 400, and 600 ppm	Increased leaf chlorosis, and kernel quality	Reduced total kernel weight and, leaf defoliation	(Khalil et al., 2015)
Pecan ( <i>Carya illinoensis</i> (Wangenh.) C. Koch)	600 ppm	Increased fruit set	Decrease in total kernel weight	(Khalil et al., 2016)
Walnuts ( <i>Juglans regia</i> L)	40, 70, and 100 mL/100 L	Significant increase in hull-dehiscence	Slight yellowing of leaves	(Lang et al., 2010)
Walnuts ( <i>Juglans regia</i> L)	0, 10, 500, and 8000 mg/L	low concentration enhances resistance and retards decay of fresh in-husk walnut	high concentration promotes senescence and decay of the fruit	(Wang et al., 2016)
Pistachos ( <i>Pistacia vera</i> L)	100 and 200 mg/L	Increased size of the nut, floral bud	Reduced number of fruit per	(Rahemi et al., 2007)

		retention, weight of kernel, and decreased % of seedless nut.	cluster	
Pistachos ( <i>Pistacia vera</i> L)	1000, 3000, and 5000 ppm	Improved cluster breaking force from the branch and endocarp removal force from the seed	-	(Atli et al., 2021)
Pistachos ( <i>Pistacia vera</i> L)	75, 150 and 200 mg/L	Decreased flower bud abscission, increased tree yield, nuts per ounce, blank and split nuts percentage improved	-	(Kamiab et al., 2020)
Cashew	50 ppm	Improved nut yield (kg), increased the number of fruit sets and reduced fruit drop	-	(Lakshmipathi et al., 2014)

## 2.5 Factors affecting the postharvest quality of Macadamia nuts

Nuts, including macadamia, are generally susceptible to oxidative rancidity and rapid deterioration due to their high oil content and fatty acid composition (Gama et al., 2018). Pre-harvest, harvest, and postharvest conditions majorly affect the physicochemical properties of nuts which in turn affect their overall quality. Pre-harvest practices among many orchards include crop management, clean-up, maturity monitoring, and harvest aids (Quinlan et al., 2008) while harvest management practices include harvest frequency, efficiency, and batch harvesting. On the other hand, the postharvest practices that affect the physicochemical properties of macadamia nuts include de-husking, sorting and resorting, dropping nuts, storage, drying, cracking, packaging, and transport (Gama et al., 2018). Other factors associated with macadamia harvesting include variations in harvest time which have been shown to affect the quality and shelf life of Macadamia nuts. Gama et al. (2020) reported that macadamia nuts harvested later in the season were found to have a shorter shelf life and lower oil quality which was demonstrated by the higher free fatty acid levels obtained compared to earlier harvested nuts. Similarly, oil extracted from early-harvested almonds exhibited superior quality, with lower K232 and K270 values and a higher K232/K270 ratio, compared to oil from late-harvested almonds (Kazantzis et al., 2003). Based on Kazantzis et al. (2003) findings, the degradation of oil quality was attributed to the increase in UV absorption coefficients K232 and K270, which are respectively linked to the formation of conjugated hydroperoxides from lipid oxidation and the presence of hydroperoxide breakdown products. On the other hand, a study on the effect of harvest time on *Torreya grandis* nuts oil quality found that later harvest nuts had lower free fatty acid levels and peroxide value compared to earlier harvested nuts (Wang et al., 2021). The findings suggest that optimal harvest timing varies depending on the nut variety and its susceptibility to lipid oxidation. The quality of all

nuts including macadamia can be revealed by various indices such as oil content, lipid oxidation, moisture content, and rancidity (Buthelezi et al., 2019). Out of all the quality indices, oil content remains the most critical quality index of macadamia because of its high unsaturated fatty acids level of more than 80 % (Rengel et al., 2015; Buthelezi et al., 2019).

## **2.6 The Possible effects of ethephon on the postharvest quality of Macadamia nuts**

The condition to which nuts are exposed before and after harvest highly affects their physicochemical compositions which is a major determinant of their quality and shelf life (Gama et al., 2018). Although ethephon application on macadamia nuts before harvest is for abscission purposes, its use has been linked with accelerated plant cell senescence (Agarwal et al., 2012). Therefore, ethylene released from ethephon could have positive or detrimental effects on nut quality. For macadamia kernels, free fatty acids (FFA) are known as a reliable quality index (Tu et al., 2021) but are highly susceptible to oxidation. Moreover, when standard acceptable limits are exceeded, they can lead to a decrease in the shelf life of the nut (Moscetti et al., 2012). Similarly, FFA is a critical quality parameter of crude palm oil (Chew et al., 2021). When ethylene from ethephon was applied to oil palm fruit bunches to induce fruit abscission, a quality improvement was observed in the form of reduced FFA and oxidative stability (Chew et al., 2021). Additionally, ethylene-treated fruits showed a significant increase in triacylglycerol (TAG), a major component of macadamia nut lipids, when compared to untreated fruits (Moreno-Pérez et al., 2011; Chew et al., 2021). Therefore, the application of ethephon on macadamia nuts could not only induce abscission but also have beneficial effects on both FFA and TAG. Standard acceptable limits of many important quality parameters such as FFA, peroxide values, and hexanal content among many have been established for various tree nuts but there is no standard for macadamia nuts due to variations in maximum acceptable limits within the species (Gama et al., 2018). The differences within

the cultivars indicate that the cultivar response to exogenous ethylene could also differ (Gama et al., 2018). Ethephon used as a postharvest treatment on fresh in-husk walnut had a positive effect on peroxide and acid value when applied at lower levels (100 and 500 mg/L) and resulted in enhancement of peroxidation of the kernel oil at higher levels of about 8000 mg/L (Wang et al., 2016). Moreover, the use of ethephon has been linked to sugar accumulation (Li et al., 2002; Wang et al., 2016). Moisture content is another critical quality parameter for macadamia nuts, which if high can have a detrimental effect on nut quality (Walton et al., 2013). Lavanya et al. (2019) reported that dip ethephon treatment of Mango (*Mangifera indica* L.) Cv. Neelum resulted in increased weight loss while (Khalil et al., 2016) reported a reduction in the total kernel weight of pecan nuts after ethephon application. Though macadamia nuts undergo drying processes, the effect of ethephon on their moisture content could be studied. However, ethephon is widely used as a harvest aid of the nut rather than as a postharvest treatment. The effects observed by various studies further motivate the investigation of the effect of ethephon on macadamia nut quality parameters (Grijalva-Contreras et al., 2011; Atli et al., 2021; Kamiab et al., 2020; Wang et al., 2016).

## **2.7 Conclusion and recommendations**

In this review, various use cases of ethephon for macadamia harvesting together with the potential effects on postharvest quality were highlighted. Here, the promising prospects of the use of ethephon to improve the quality of macadamia nuts are underscored. Although there is limited literature on this positive use of ethephon, the knowledge can be extracted from understanding the underlying mechanism of action. One of the challenges associated with ethephon use is its impact on the senescence of tissues and organs, which is the major factor for the acceleration of the aging or ripening process of macadamia nuts (Rademacher, 2015). Putting this into perspective, future research should therefore focus on investigating

the mechanisms underlying the senescence process in ethephon-treated macadamia nuts and developing strategies to counteract or delay senescence. This strategy will preserve the kernel quality and extend the shelf life of the ethephon treated nuts. Additionally, applying an ethylene inhibitor on nuts harvested from ethephon-treated trees can help slow down the senescence process and maintain the quality of the nuts for a longer duration (Rademacher, 2015). Research also suggests the possibility of cultivar-specific differences in the level of response/sensitivity to ethephon application (Lang et al., 2010). However, there is a lack of comprehensive trial experiments examining the effects of ethephon on different macadamia cultivars (Trueman et al., 2002). Nevertheless, future research should aim to explore the differential response of a wide range of commercial macadamia nut cultivars to ethephon application. By conducting cultivar-specific studies, valuable insights can be obtained from the optimal ethephon application rates and timing for different cultivars, leading to improved overall nut quality.

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### **CHAPTER 3: IMPACT OF PREHARVEST ETHEPHON FOLIAR SPRAY ON THE POSTHARVEST FATTY ACID PROFILE AND DIETARY INDICATORS OF MACADAMIA NUTS**

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### 3.1 Abstract

The use of ethephon, designed to stimulate nut detachment, initiates the release of ethylene, a well-established regulator of postharvest shelf-life in various agricultural products. This study aimed to assess the impact of ethephon application on individual fatty acids and dietary indicators in two macadamia nut cultivars, namely '788' and 'Beaumont,' during postharvest storage. Nuts that naturally abscised and those detached through ethephon treatment were divided into two groups: the ethylene-treated group (ED) and the control group (CD). Nuts were stored at 25 °C and sampled at 0, 36, and 72 days for fatty profile analysis. Our findings indicated a significant increase in stearic acid content in ED nuts (24,622 µg/g) compared to CD nuts (16,764 µg/g) at the end of the storage period for the 'Beaumont' cultivar. Additionally, unsaturated fatty acids (USFAs), such as eicosatrienoic acid + erucic acid (C20:3n3 + C22:1) and eicosatrienoic acid + alpha-linolenic acid (C20:1 + C18:3n3), were notably reduced. Hierarchical clustering analysis revealed positive correlations between ethylene treatment and saturated fatty acids (SFAs) in both 'Beaumont' (0.78) and '788' (0.80) cultivars. This also coincided with an increase in atherogenic indices, thrombogenic index, and saturation index and a decrease in the hypocholesterolemic/hypercholesterolemic ratio and arachidonic acid (C20:4n6) within the ED group of the 'Beaumont' cultivar, collectively potentially impacting nutritional quality negatively. Furthermore, our findings indicated that the PUFA:SFA ratio was higher in CD (0.51) compared to ED (0.45) on day 72 for the 'Beaumont' cultivar, revealing differences in fatty acid compositions between the two treatment groups. Conversely, for '788', both ED and CD had a PUFA:SFA ratio below 0.45, indicating an increased risk of cardiovascular diseases. These results suggest that ethephon treatment increases SFA levels and reduces USFA levels in the 'Beaumont' cultivar, while the response to ethylene varies between the two cultivars. Thus, the study provides insight into

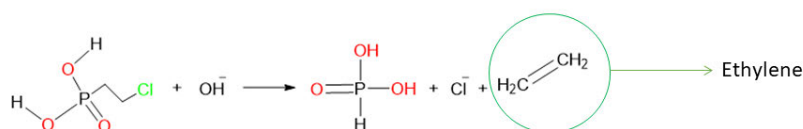
the significant role of modifying ethephon treatment methods and careful cultivar selection in the attainment of optimal nutritional value and shelf-life of macadamia nuts.

**Keywords:** *ethephon; preharvest; postharvest; ethylene; fatty acids; macadamia; storage*

### **3.2 Introduction**

Macadamia nuts, specifically *Macadamia integrifolia* and *Macadamia tetraphylla*, are highly sought after due to their nutritional value and rank among the most economically profitable crop varieties in subtropical regions across the globe [1]. With approximately 75.4 g of fat per 100 g of nuts, macadamia nuts are known for their high fat content [2], which consists of various fatty acids categorized based on the number of double bonds in their carbon chain [3]. These fatty acids fall into three groups: saturated fatty acids (SFAs) if there are no double bonds, monounsaturated fatty acids (MUFAs) if there is one double bond, and polyunsaturated fatty acids (PUFAs) if there is more than one double bond [3]. Notably, MUFAs form the predominant component of fat in macadamia nuts, comprising up to 54% of the total fat content [4]. Ethephon, an ethylene releasing compound (Figure 1), is commonly used to promote nut abscission during macadamia nut harvesting. This is essential due to the extended nut drop experienced in macadamia, leading to challenges such as multiple harvest periods that incur additional costs and labour-intensive operations [5]. As a natural plant hormone, ethylene plays a crucial role in various physiological processes, which include fruit ripening, senescence, and abscission [6,7]. The fat content of nuts is also correlated with fruit ripening whereby mature crops exhibit a higher ratio of unsaturated fatty acids to saturated fatty acids compared to immature crops [8]. Furthermore, studies have demonstrated the impact of ethylene on fatty acid metabolism. For instance, exogenous ethylene fumigation accelerated the ripening of oil palm fruit and reduced free fatty acid (FFA) levels [9]. Another

study reported that ethephon treatment increased the expression of glycerol-3-phosphate acyltransferase (GPAT) in Kentucky bluegrass, resulting in elevated unsaturated fatty acid levels [10]. Moreover, Li [11] found that ethephon treatment led to an upregulation of MdCER6, which is a pivotal gene responsible for the synthesis of ultra-long-chain fatty acids in ‘Starkrimson’ apples [11].



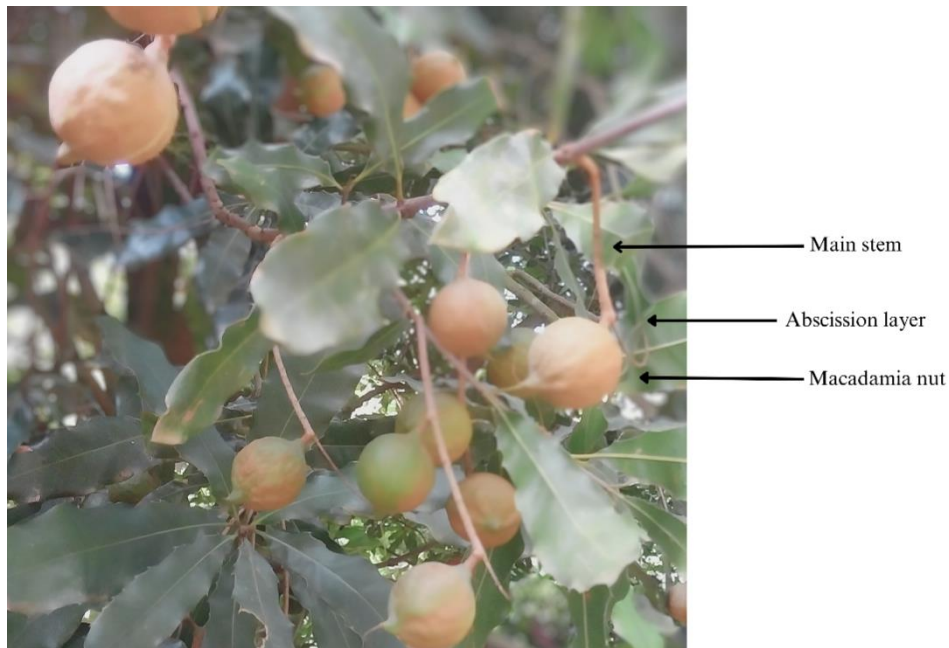
**Figure 3.1** Chemical structure equation depicting the breakdown of ethephon and the release of ethylene

Given ethylene’s significant role in the regulation of postharvest shelf-life for various agricultural products [12], it becomes essential to assess its effect on fatty acids and dietary indicators in macadamia nuts as they serve as a critical quality parameter for both edible nuts and oils [13]. Therefore, this study aims to examine how ethephon treatment affects individual fatty acids and dietary indicators in macadamia nuts that abscised due to the treatment as well as those that naturally abscised during postharvest storage. By investigating the impact of ethephon on fatty acid composition, this research aims to contribute valuable insights into the postharvest quality of macadamia nuts and their suitability for consumption and various applications.

### 3.3 Materials and methods

#### 3.3.1 Ethephon Spray and Concentration

Macadamia nut cultivars ‘Beaumont’ and ‘788’ were harvested from Fyvie estates, uMlaas Road, Camperdown, KwaZulu-Natal, South Africa (Latitude: 29°47′50.3″ S, Longitude: 30°27′54″ E). The area is characterized by well-drained sandy loam soil, and a range of cultural practices have been implemented to optimize macadamia nut production. These practices encompass drip irrigation for maintaining consistent moisture levels, customized fertilization based on annual soil testing, integrated pest management techniques, regular pruning aimed at enhancing canopy structure and air circulation, careful harvesting at the ideal stage of maturity, and precise application of ethephon to specific macadamia trees to facilitate abscission. Ethylene was administered as ethephon on physiologically mature nuts, serving as a vital plant growth regulator to facilitate nut abscission in macadamia trees [14] (Figure 2). The concentration of ethephon was determined in accordance with industry standards. For ‘788’ macadamia trees, 4 L of ethephon 480 SL® per hectare was mixed with 3000 L of water per hectare, resulting in a concentration of 13.33 mL/L. ‘Beaumont’ macadamia trees received a dosage of 5 L of ethephon 480 SL per hectare combined with 3000 L of water per hectare, resulting in a concentration of 16.67 mL/L. Both doses were applied using a Cima mist blower to promote nut abscission. The application of ethephon specifically targeted physiologically mature nuts, and those that naturally detached and fell to the ground were considered successfully abscised nuts.



**Figure 3.2** Diagram depicting the role of ethephon in the abscission process of macadamia nuts: stimulated abscission layer for facilitating detachment.

### ***3.3.2 Nut Collection and Preparation***

Freshly harvested nuts, with an initial moisture content of approximately 20.61% on a dry basis (d.b.), included those that had fallen from both ethephon-treated and non-ethephon-treated trees. Nuts with green husks were directly picked from the ground, representing both the ethephon-treated (ED) and control (CD) groups. Following harvesting, the fruits were mechanically dehusked within 24 h, weighed, and subsequently dried to approximately 2.42% on a dry basis (d.b.) using a laboratory Memmert U15 oven from Germany. The drying process involved temperatures starting at 35 °C on the first day, 38 °C on the second day, and 50 °C on the third day, following a modified version of the method outlined by Buthelezi [15]. Following drying nuts were cracked.

### ***3.3.3 Storage Conditions***

In this research study, a total of 150 macadamia kernels were used for each of the two cultivars ('788' and 'Beaumont'). Within each cultivar, these kernels were divided into two distinct groups: 75 kernels originated from trees subjected to ethephon treatment (ED), representing the treated group, while the remaining 75 kernels were from control trees (CD), which remained untreated. Subsequently, these kernels were organized into three separate replicates each comprising 5 kernels and securely enclosed within conventional polythene bags. These bags, containing the kernels, were then stored at a temperature of 25 °C for a maximum duration of 72 days. The selected storage duration was chosen to simulate accelerated shelf-life studies, which involve elevated storage conditions, frequently with higher temperatures in order to predict how long the product will remain suitable for consumption or use when stored under standard storage conditions [15]. To ensure a comprehensive evaluation of the fatty acid profile, a systematic sampling regimen was implemented, with sampling events conducted at predetermined intervals of 0, 36, and 72 days. These time points were selected to capture any potential dynamics in the fatty acid composition throughout the storage period. After each sampling event, the collected macadamia nuts were preserved at -20 °C, maintaining the integrity and quality of the samples for subsequent in-depth analysis.

### ***3.3.4 Quantification of Fatty Acid Profile***

The fatty acid analysis was conducted following modification of the method described by [4,16]. Initially, 100 mg of the sample was weighed, and 2 mL of hexane was added. As an internal standard, 50 µL of 1000 ppm heptadecanoic acid (C17) was also added. The mixture received 1 mL of 20% sulfuric acid in a methanol solution and was then incubated in an oven at 80 °C for one hour. After incubation, the mixture was allowed to cool to room temperature.

To extract the fatty acid methyl esters (FAMES), 3 mL of 20% (w/v) NaCl was added, and the materials were agitated firmly for phase separation. The upper hexane phase containing FAMES was transferred to a gas chromatograph (GC) vial. FAMES were separated using a GC (Trace1300, Thermo Scientific, Austin, TX, USA) connected to a flame ionization detector (FID) on a non-polar Stabil wax column (60 m, 0.32 mm ID, 0.25  $\mu$ m film thickness). Helium at 1 mL/min was employed as the carrier gas, and the injector temperature was maintained at 240 °C. A 5:1 split ratio was used for injecting 1  $\mu$ L of the material. The oven temperature was programmed as follows: 50 °C for 2 min, increased to 180 °C at a rate of 25 °C per minute and held for 2 min, 200 °C at a rate of 3 °C per minute and held for 5 min, and finally 240 °C at a rate of 4 °C per minute and kept for 15 min. Identification of each FA in the chromatogram was performed by comparing the retention times with certified standard mixes (Grain FAME Mix Supelco, Bellefonte, PA, USA; Catalog No: 47801) using the Chrom-Card data system version 2.3 software for Windows (Thermo Electron, Rodano, Italy). The quantification of FAs was achieved by measuring the peak areas on the chromatogram and expressing the results in grams of FA per gram of total FAs. The obtained fatty acid composition was used to calculate the total fatty acids (TFAs), the ratio of saturated to unsaturated fatty acids (SFA/MUFA), and the ratio of monounsaturated to polyunsaturated fatty acids (MUFA/PUFA).

### ***3.3.5 Dietary Indicators***

The dietary indices of macadamia nuts, encompassing the atherogenic index, thrombogenic index, saturation index, lipid nutritional value, and the hypocholesterolemic/hypercholesterolemic ratio, were computed using the equations described by [17], as presented below:

$$AI = \frac{C\ 12:0 + 4 \times C\ 14:0 + C\ 16:0}{MUFA + n-6 + n-3} \quad (1)$$

$$TI = \frac{C\ 14:0 + C\ 16:0 + C\ 18:0}{0.5\ MUFA + 0.5\ n-6 + 3\ n-3 + \frac{n-3}{n-6}} \quad (2)$$

$$\frac{S}{p} = \frac{C\ 14:0 + C\ 16:0 + C\ 18:0}{MUFA + PUFA} \quad (3)$$

$$NV = \frac{C\ 12:0 + C\ 14:0 + C\ 16:0}{C\ 18:1 + C\ 18:2} \quad (4)$$

$$\frac{h}{H} = \frac{C\ 16:1 + C\ 18:1 + C\ 18:2 + C\ 20:4\ n-6}{C\ 12:0 + C\ 14:0 + C\ 16:0} \quad (5)$$

### 3.3.5 Data Analysis

Analysis of variance (ANOVA) was performed using GenStat® 20th Edition (VSN International Ltd., Hertfordshire, UK). Means were separated using the least significant difference (LSD) test at 5% levels of significance. Principal component analysis (PCA), biplot diagrams, and hierarchical clustering heat map analysis were utilized in Python programming to comprehensively identify the impact of ethephon on the fatty acid profile of the two macadamia nut cultivars during postharvest storage.

## 3.4 Results and discussion

### 3.4.1 Fatty Acid Profile

Table 3.1 and Table 3.2 present the results of fatty acid methyl ester (FAME) analysis from Beaumont' and '788' cultivars after 72 days of storage at 25 °C. The concentration of monounsaturated was the most abundant in both cultivars, particularly oleic acid (C18:1) and palmitoleic acid (C16:1) (see Table 1 and Table 2). Macadamia nuts are well known for their abundance of monounsaturated fatty acids (MUFAs) [4,18]. The concentration of oleic acid

(C18:1) was not significantly affected by ethephon or storage time. However, there was a significant difference ( $p < 0.001$ ) in their concentrations between the two cultivars, with 'Beaumont' exhibiting the highest concentration. Palmitoleic acid (C16:1) was influenced by the interaction effect of Treatment  $\times$  Day  $\times$  Cultivar ( $p < 0.001$ ); particularly in the 'Beaumont' cultivar, on day 0, ED (156,446  $\mu\text{g/g}$ ) exhibited a higher concentration of palmitoleic acid compared to CD (99,582  $\mu\text{g/g}$ ), whereas in '788' CD displayed a higher concentration than ED. Macadamia nuts are also known for their low levels of saturated fatty acids (SFAs), with palmitic acid (C16:0) and stearic acid (C18:0) being the most abundant SFAs [18], as indicated in Table 3.1 and Table 3.2.

**Table 3.1** The fatty acid compositions ( $\mu\text{g/g}$ ) of ‘Beaumont’ macadamia kernels from the ethylene-treated group (ED) and the control group (CD) during postharvest storage.

Treatment Day		Lauric Acid (C12:0)	Myristic Acid (C14:0)	Pentadecanoic Acid (C15:0)	Palmitic Acid (C16:0)	Palmitoleic Acid (C16:1)	Heptadecanoic Acid (C17:0)	Stearic Acid (C18:0)
CD	0	395.1 ± 18.08 ab	3755 ± 130.79 abc	70.02 ± 4.10 ab	56838 ± 2561.39 abc	99582 ± 4990.18 ab	162.9 ± 11.19 a	19291 ± 658.01 abc
ED	0	437.6 ± 16.25 ab	4566 ± 317.24 bcd	90.53 ± 9.11 b	68632 ± 4236.48 c	156446 ± 9299.51 e	219.8 ± 8.08 a	21872 ± 1652.29 bc
CD	36	462.3 ± 27.09 ab	4815 ± 107.35 cd	75.04 ± 10.54 ab	62736 ± 1156.96 bc	136538 ± 3511.92 de	213.4 ± 16.08 a	19178 ± 107.52 abc
ED	36	442.4 ± 5.05 ab	4382 ± 9.46 bcd	77.38 ± 2.38 ab	63297 ± 669.35 bc	137833 ± 1508.76 de	213.4 ± 0.91 a	18265 ± 164.54 ab
CD	72	592.5 ± 12.29 c	5165 ± 51.19d	70.76 ± 1.83 ab	56628 ± 1306.99 abc	134027 ± 2922.03 d	184.0 ± 8.86 a	16764 ± 170.26 ab
ED	72	389.4 ± 4.85 ab	4210 ± 83.21bcd	75.91 ± 2.62 ab	62674 ± 1034.34 bc	118159 ± 2807.36 bcd	221.7 ± 37.56 a	24622 ± 658.74 c
Treatment Day		Oleic Acid C18:1 (cis)	Linoleic Acid C18:2 (cis)	Arachidic Acid (C20:0)	Eicosatrienoic Acid + Alpha-linolenic Acid (C20:1 + C18:3n3)	Heneicosanoic Acid (C21:0)	Docosanoic Acid (C22:0)	Eicosatrienoic Acid + Erucic Acid (C20:3n3+C22:1)
CD	0	342102 ± 10,793.36 abc	19530 ± 798.08 f	15283 ± 587.35 ab	7279 ± 209.79 bcd	35.44 ± 6.34 ab	4176 ± 101.99 abc	737.4 ± 15.42 cd
ED	0	378430 ± 25,126.64 bc	19290 ± 1146.71 ef	17771 ± 1324.36 b	7559 ± 492.45 cd	55.98 ± 7.79 b	5137 ± 203.35 c	726.5 ± 45.03 cd
CD	36	359739 ± 5505.98 abc	18095 ± 439.15 ef	15595 ± 365.73 ab	7422 ± 160.33 cd	42.76 ± 2.39 ab	4858 ± 240.69 bc	732.9 ± 17.59 cd
ED	36	352704 ± 2704.59 abc	18764 ± 210.28 ef	15151 ± 113.74 ab	7465 ± 100.44 cd	47.85 ± 5.16 ab	4865 ± 167.58 bc	783.2 ± 13.18 de
CD	72	387473 ± 4298.73 c	15316 ± 471.14 cde	15252 ± 119.84 ab	8739 ± 84.61 d	47.37 ± 2.85 ab	4811 ± 177.05 abc	938.8 ± 10.98 e
ED	72	346413 ± 4826.80 abc	17173 ± 442.70 def	17128 ± 378.03 b	6637 ± 117.11 abc	41.35 ± 2.79 ab	4366 ± 189.99 abc	641 ± 13.15 bcd
Treatment Day		Arachidonic Acid (C20:4n6)	Tricosanoic Acid (C23:0)	Tetracosanoic Acid (C24:0)	MUFA	PUFA	SFA	PUFA: SFA
CD	0	31.53 ± 4.42 a	68.46 ± 3.68 a	1924 ± 45.91 bcde	441684 ± 15,772.48 abc	42860 ± 1585.13 de	80512 ± 3342.11 abcd	0.53 ± 0.01 g
ED	0	59.27 ± 14.13 ab	48.25 ± 6.97 a	2020 ± 129.79 de	534876 ± 34,417.53 c	45406 ± 2994.74 e	95818 ± 6208.41 d	0.47 ± 0 de
CD	36	46.68 ± 6.27 a	55.12 ± 1.71 a	1912 ± 43.31 bcde	496278 ± 8416.62 abc	41891 ± 958.29 cde	87480 ± 1366.18 bcd	0.48 ± 0 e
ED	36	56.5 ± 2.71 a	55.66 ± 0.70 a	2027 ± 29.26 de	490537 ± 4113.63 abc	42219 ± 424.29 cde	86677 ± 846.50 bcd	0.49 ± 0 ef
CD	72	114.8 ± 26.09 b	52.59 ± 2.89 a	2137 ± 29.270 e	521500 ± 5037.15 bc	40360 ± 619.07 bcde	79404 ± 1529.48 abcd	0.51 ± 0 fg
ED	72	26.38 ± 4.56 a	54.88 ± 1.69 a	1983 ± 47.90 cde	464572 ± 7351.12 abc	41605 ± 931.15 cde	92193 ± 1768.74 cd	0.45 ± 0 bcd
Treatment Day		TFA	Omega-6 (n-6)	Omega-3 (n-3)	$\sum$ n-6)/( $\sum$ n-3)			
CD	0	571240 ± 12,614.22 abc	19350 ± 797.97 e	8016 ± 225.19 bc	2.44 ± 0.03 e			
ED	0	683381 ± 450,303.65 c	19561 ± 1160.79 e	8285 ± 537.43 bc	2.31 ± 0.03 de			
CD	36	632,517 ± 20,433.35 abc	18141 ± 439.6 e	8155 ± 177.72 bc	2.21 ± 0.02 d			

ED	36	626429 ± 4658.12 abc	18820 ± 211.31 e	8249 ± 113.50 bc	2.28 ± 0.02 de
CD	72	648314 ± 13,223.65 bc	15431 ± 474.97 cde	8155 ± 94.61 bc	1.59 ± 0.05 c
ED	72	604813 ± 13,609.36 abc	17199 ± 444.30 de	7278 ± 130.23 ab	2.36 ± 0.04 de

CD (control drop), ED (ethephon drop)  $\Sigma$  (sum), SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), PUFA: SFA (polyunsaturated fatty acids: saturated fatty acids), TFA (total fatty acids). Values are the mean  $\pm$  SE. Lowercase letters (a–g) indicate differences between treatments during storage.

**Table 1.2** The fatty acid compositions ( $\mu\text{g/g}$ ) of ‘788’ macadamia kernels from the ethylene-treated group (ED) and the control group (CD) during postharvest storage.

Treatment	Day	Lauric Acid (C12:0)	Myristic Acid (C14:0)	Pentadecanoic Acid (C15:0)	Palmitic Acid (C16:00)	Palmitoleic Acid (C16:1)	Heptadecanoic Acid (C17:0)	Stearic Acid (C18:0)
CD	0	456.1 ± 52.38 ab	3856 ± 560.64 abc	58.21 ± 5.83 a	49028 ± 6394.12 ab	116149 ± 15516.71 bcd	241.3 ± 39.24 a	18472 ± 2361.10 ab
ED	0	340.1 ± 20.96 a	2704 ± 140.84 a	49.53 ± 1.21 a	41870 ± 2049.04 a	93446 ± 5644.47 a	219.2 ± 8.93 a	16904 ± 1051.49 ab
CD	36	362.4 ± 14.82 a	3540 ± 160.33 ab	67.17 ± 5.16 ab	49733 ± 1705.95 ab	105114 ± 2412.54 abc	297.0 ± 40.63 a	14310 ± 617.25 a
ED	36	441.5 ± 28.44 ab	3793 ± 212.98 abc	68.13 ± 6.77 ab	48819 ± 2740.12 ab	122610 ± 5727.06 cd	231.7 ± 17.53 a	18092 ± 865.77 ab
CD	72	491.3 ± 9.31 bc	4018 ± 134.20 bcd	54 ± 1.71 a	44103 ± 1424.11 a	111163 ± 4861.47 abcd	289.5 ± 60.94 a	19711 ± 124.08 abc
ED	72	394.6 ± 6.71 ab	3362 ± 98.02 ab	52.14 ± 1.14 a	45678 ± 1431.26 a	103515 ± 2746.10 abc	208.8 ± 9.35 a	21783 ± 766.09 bc
Treatment	Day	Oleic Acid C18:1 (cis)	Linoleic Acid C18:2 (cis)	Arachidic Acid (C20:0)	Eicosatrienoic Acid + Alpha-linolenic Acid (C20:1 + C18:3n3)	Heneicosanoic Acid (C21:00)	Docosanoic Acid (C22:00)	Eicosatrienoic Acid + Erucic Acid (C20:3n3+C22:1)
CD	0	297263 ± 41541.23 abc	11257 ± 1723.93 abc	14227 ± 1899.80 ab	6191 ± 888.11 abc	27.53 ± 6.58 ab	4146 ± 566.59 abc	598.8 ± 90.53 abcd
ED	0	290300 ± 8623.62 ab	8630 ± 315.15 a	12548 ± 416.73 a	6071 ± 162.57 abc	22.69 ± 0.47 a	3581 ± 133.90 a	595.5 ± 21.31 abcd
CD	36	301545 ± 10891.06 abc	13898 ± 585.06 bcd	11641 ± 405.32 a	5800 ± 231.48 abc	28.36 ± 4.54 ab	3632 ± 129.58 ab	503.8 ± 12.77 ab
ED	36	287033 ± 18356.90 ab	10086 ± 441 ab	13709 ± 744.47 ab	6241 ± 392.35 abc	28.14 ± 7.28 ab	3707 ± 268.38 ab	582.3 ± 37.33 abc
CD	72	282001 ± 8719.03 a	7938 ± 375.11 a	14415 ± 182.85 ab	5250 ± 156.46 a	20.79 ± 3.05 a	3795 ± 58.76 ab	468.3 ± 15.66 ab
ED	72	284023 ± 9958.49 a	7508 ± 220.39 a	14021 ± 570.28 ab	5301 ± 218.14 ab	34.69 ± 9.14 ab	3571 ± 163.07 a	430.6 ± 29.59 a
Treatment	Day	Arachidonic Acid (C20:4n6)	Tricosanoic Acid (C23:0)	Tetracosanoic Acid (C24:0)	MUFA	PUFA	SFA	PUFA: SFA

CD	0	23.1 ± 9.94 a	32.99 ± 5.69 a	1573 ± 225.10 abcd	413412 ± 57056.50 abc	32297 ± 4592.10 abcd	72112 ± 9394.56 abc	0.45 ± 0.01 bc
ED	0	8.29 ± 42.10 a	33.79 ± 0.52 a	1518 ± 48.96 abcd	383745 ± 14244.28 a	27853 ± 875.29 a	62086 ± 3233.89 a	0.45 ± 0.01 bcd
CD	36	12.77 ± 4.57 a	45.92 ± 19.07 a	1392 ± 58.131 a	392147 ± 13243.63 ab	30647 ± 1180.94 ab	68309 ± 249.77 ab	0.49 ± 0 ef
ED	36	28.33 ± 14.98 a	50.43 ± 3.31 a	1574 ± 109.31 abcd	424156 ± 24051.43 abc	31855 ± 1615.99 abc	71446 ± 3864.65 abc	0.47 ± 0 cde
CD	72	10.18 ± 3.20 a	36.7 ± 1.51 a	1464 ± 38.19 ab	395186 ± 13574 ab	27686 ± 625.17 a	69407 ± 1482.83 ab	0.39 ± 0 a
ED	72	7.97 ± 4.73 a	38.89 ± 4.03 a	1473 ± 59.37 abc	385517 ± 12657.02 a	27663 ± 1012.57 a	70739 ± 2287.67 abc	0.40 ± 0 a
<b>Treatment</b>	<b>Day</b>	<b>TFA</b>	<b>Omega-6 (n-6)</b>	<b>Omega-3 (n-3)</b>	<b>∑ n-6)/(∑ n-3)</b>			
CD	0	523601 ± 97815 abc	11280 ± 1733.68 abc	6790 ± 978.47 ab	1.66 ± 0.003 c			
ED	0	478839 ± 28603.60 a	8638 ± 313.04 a	6667 ± 183.87 ab	1.3 ± 0.03 a			
CD	36	497414 ± 31833.44 ab	13910 ± 580.49 bcd	6304 ± 235.52 ab	2.21 ± 0.04 d			
ED	36	531603 ± 28788.47 abc	10115 ± 455.94 ab	6824 ± 429.62 ab	1.49 ± 0.03 bc			
CD	72	498714 ± 11740.23 ab	7948 ± 378.29 a	5718 ± 172.10 a	1.39 ± 0.02 ab			
ED	72	487919 ± 24421 ab	7516 ± 222.80 a	5732 ± 233.23 a	1.30 ± 0.02 a			

CD (control drop), ED (ethephon drop) ∑ (sum), SFA (saturated fatty acid), MUFA (monounsaturated fatty acid), PUFA (polyunsaturated fatty acid), PUFA: SFA (polyunsaturated fatty acid: saturated fatty acid), TFA (total fatty acid). Values are the mean ± SE. Lowercase letters (a–f) indicate differences between treatments during storage.

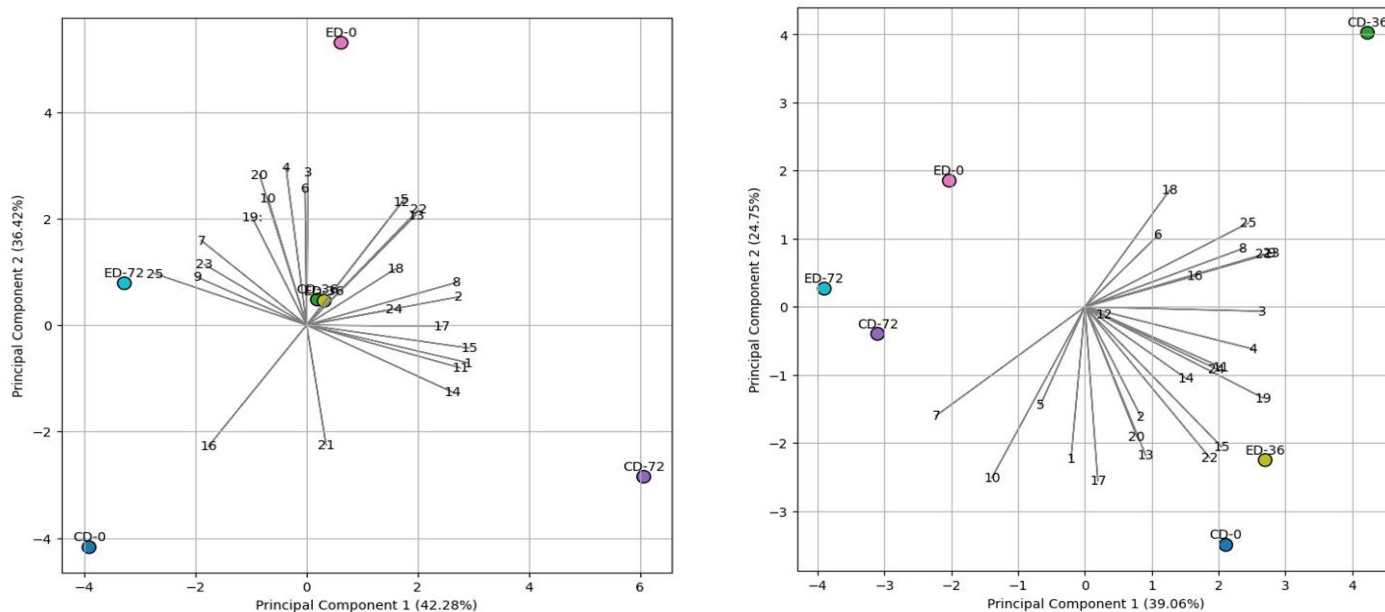
In the 'Beaumont' cultivar, after 72 days of storage, the concentration of palmitic acid was found to be higher in the ED (62,674  $\mu\text{g/g}$ ) harvest compared to the CD (56,628  $\mu\text{g/g}$ ) harvest, although this difference was not statistically significant. Nuts from the ED group exhibited a significantly higher stearic acid (C18:0) content at 24,622  $\mu\text{g/g}$  compared to the CD group, which had 16,764  $\mu\text{g/g}$ . In the '788' cultivar, no significant differences in palmitic acid and stearic acid were observed at the end of the storage period. Stearic acid is a long-chain saturated fatty acid known for its association with reductions in low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and Apolipoprotein A-I levels, as well as an increase in Cholesteryl ester transfer protein mass [19,20]. Moreover, stearic acid is known for its effects on HDL cholesterol levels and holds considerable importance within dietary recommendations due to its potential to influence the crucial ratio of HDL cholesterol to LDL cholesterol, which is a key factor in determining cardiovascular health [21]. These findings are consistent with previous studies by [22], which also reported an increase in saturated fatty acids and a decrease in unsaturated fatty acids in response to ethylene treatment [22]. Another study by [23] also reported that the use of ethylene may reduce unsaturated fatty acids. In this study, we observed a significant decrease in unsaturated fatty acids in ED, particularly Eicosatrienoic acid + Erucic acid (C20:3n3 + C22:1) and Eicosatrienoic acid + Alpha-linolenic acid (C20:1 + C18:3n3). These changes in fatty acid composition may be due to the effect of ethylene on regulating genes associated with fatty acid biosynthesis [24]. A study by Li [8] found that preharvest ethylene treatment in *Camellia oleifera* increased the alpha-linolenic acid content by regulating genes involved in linoleic acid and  $\alpha$ -linolenic acid metabolism [8]. For eicosatrienoic acid + alpha-linolenic acid (C20:1 + C18:3n3), the interaction effect of Treatment  $\times$  Day  $\times$  Cultivar on this combination was significant ( $p < 0.05$ ), with CD (8739  $\mu\text{g/g}$ ) displaying a higher content compared to ED (6637  $\mu\text{g/g}$ ) (Table

3.1). Moreover, for eicosatrienoic acid + erucic acid (C20:3n3 + C22:1), the interaction effect of Treatment × Day × Cultivar on this combination was significant ( $p < 0.05$ ), with CD (938.8) displaying a higher content compared to ED (641 µg/g) (Table 3.1). In the ‘788’ cultivar, no significant differences were observed in eicosatrienoic acid + erucic acid (C20:3n3 + C22:1) between treatments or during storage (Table 3.2). Unsaturated fatty acids offer various health benefits when incorporated into the diet [25]. However, erucic acid (C22:1) is a monounsaturated omega-9 fatty acid found in numerous plant oils [26]. It is not easily digested and absorbed in the human body, and its potential health risks have led to strict guidelines by regulatory bodies on maximum erucic acid content in oils [27]. Furthermore, at the end of the storage period, the content of arachidonic acid (C20:4n6), a type of polyunsaturated fat, was influenced by the interaction of Treatment × Day × Cultivar ( $p < 0.001$ ), with ED (26.38 µg/g) showing a significantly lower content compared to CD (114.8 µg/g) (Table 3.1). In the ‘788’ cultivar, no significant differences were observed in arachidonic acid (C20:4n6) between treatments or during storage (3.2). Ros [28] reported that MUFAs or n-6 PUFAs, such as arachidonic acid (C20:4n6), lead to lower blood cholesterol levels and potentially provide beneficial effects on inflammation, thrombosis, and vascular reactivity [28]. Moreover, in the ‘Beaumont’ cultivar, CD (0.51) had a significantly higher PUFA:SFA ratio than ED (0.45) at day 72, indicating distinct fatty acid compositions between the two groups. As discussed earlier, ethephon is known to induce an increase in saturated fatty acids and a decrease in unsaturated fatty acids, which could lead to an overall reduction in the PUFA:SFA ratio. A high PUFA/SFA ratio is recommended due to its health benefits, whereas a PUFA/SFA ratio lower than 0.45 may increase the incidence of cardiovascular diseases [29]. For the ‘Beaumont’ cultivar, all of the nuts had a PUFA/SFA that was equal to or higher than 0.45. A similar trend was observed for the ‘788’, except for on day 72, where both ED and CD were lower than 0.45.

In the 'Beaumont' cultivar, the  $(\sum n-6)/(\sum n-3)$  ratio remained consistent for both ED and CD treatments from day 0 to 36. However, by day 72, CD exhibited the lowest ratio (1.59) compared to ED (2.36), suggesting changes in the  $(\sum n-6)/(\sum n-3)$  ratio over the storage period. This decrease in the  $(\sum n-6)/(\sum n-3)$  ratio of CD at day 72 was also observed in cultivar '788'. However, the  $(\sum n-6)/(\sum n-3)$  ratio of ED was lower for day 0 and 36 in '788'. The  $(\sum n-6)/(\sum n-3)$  represents the ratio of the total omega-6 (n-6) polyunsaturated fatty acids (PUFAs) to the total omega-3 (n-3) polyunsaturated fatty acids (PUFAs) [30]. A lower ratio of omega-6 to omega-3 PUFAs is believed to have health benefits and may contribute to better overall health and reduced risk of diseases such as nonalcoholic fatty liver disease [31]. A low n-6 to n-3 polyunsaturated fatty acid (PUFA) ratio of 4:1 was found to result in a significant reduction of approximately 30% in hepatic fat content among obese youth with nonalcoholic fatty liver disease [32]. For both cultivars, the  $(\sum n-6)/(\sum n-3)$  ratio was found to be within the acceptable low ratio of 4:1 or lower.

#### **3.4.2 Principal Component Analysis (PCA)**

Principal component analysis has been used as a statistical analysis method for data reduction in various data analyses [33]. In this study, it was employed to identify the most relevant fatty acid variables and their correlations with specific treatments, providing insights into the impact of treatments on the fatty acid composition of the nuts. For the '788' cultivar, PC1 accounted for 39.06%, and PC2 accounted for 24.75% of the total variability (Figure 3.3b). In the PCA analysis of the '788' cultivar, the influence of the ethephon treatment was observed in the ED-36 group, where it showed a close association with PUFAs (Figure 3.3b). For the 'Beaumont' cultivar, PC1 accounted for 43.28%, and PC2 accounted for 36.42% of the total variability (Figure 3.3a).



**Figure 3.3** The biplot of Principal Component 1 (PC1) versus Principal Component 2 (PC2) illustrates the relationship between fatty acids and different treatments for (a) 'Beaumont' and (b) '788' cultivars during storage. The treatments are denoted as CD for control drop and ED for ethephon drop, and the storage days are represented as 0, 36, and 72. Specific numerical representations were assigned to various fatty acid components for clarity and ease of reference. These numerical assignments are as follows: 1 represents C12, 2 represents C14, 3 represents C15, 4 represents C16, 5 represents C16:1, 6 represents C17, 7 represents C18, 8 represents C18:1 (cis), 9 represents C18:2 (cis), 10 represents C20, 11 represents C20:1 + C18:3n3, 12 represents C21, 13 represents C22, 14 represents C20:3n3+C22:1, 15 represents C20:4n6, 16 represents C23, 17 represents C24, 18 represents PUFA (polyunsaturated fatty acid), 19 represents SFA (saturated fatty acid), 20 represents PUFA:SFA, 21 represents TFA =  $\sum$  (Sum of all fatty acids), 22 represents Omega-6 (n-6), 23 represents Omega-3 (n-3), and 24 represents  $\frac{\sum n-6}{(\sum n-3)}$ .

The  $(\sum n-6)/(\sum n-3)$  ratio and stearic acid (C18:0) exhibited a positive correlation with the ED-72 treatment, as indicated in Figure 3a. On the other hand, eicosatrienoic acid + erucic acid (C20:3n3 + C22:1), arachidonic acid (C20:4n6), lauric acid (C12:0), and PUFA:SFA ratio vectors pointed toward the CD-72 treatment, implying a positive correlation, as shown in Figure 3.3a. These results are also consistent with the findings presented in Table 3.1 and Table 3.2. Figure 3.3. The biplot of Principal Component 1 (PC1) versus Principal Component 2 (PC2) illustrates the relationship between fatty acids and different treatments for (a) 'Beaumont' and (b) '788' cultivars during storage. The treatments are denoted as CD for control drop and ED for ethephon drop, and the storage days are represented as 0, 36, and 72. Specific numerical representations were assigned to various fatty acid components for clarity and ease of reference. These numerical assignments are as follows: 1 represents C12, 2 represents C14, 3 represents C15, 4 represents C16, 5 represents C16:1, 6 represents C17, 7 represents C18, 8 represents C18:1 (cis), 9 represents C18:2 (cis), 10 represents C20, 11 represents C20:1 + C18:3n3, 12 represents C21, 13 represents C22, 14 represents C20:3n3 + C22:1, 15 represents C20:4n6, 16 represents C23, 17 represents C24, 18 represents PUFA (polyunsaturated fatty acid), 19 represents SFA (saturated fatty acid), 20 represents PUFA:SFA, 21 represents TFA =  $\sum$  (Sum of all fatty acids), 22 represents Omega-6 (n-6), 23 represents Omega-3 (n-3), and 24 represents  $\sum n-6)/(\sum n-3)$ .

### ***3.4.3. Hierarchical Clustering Heat Map and Correlation Analysis***

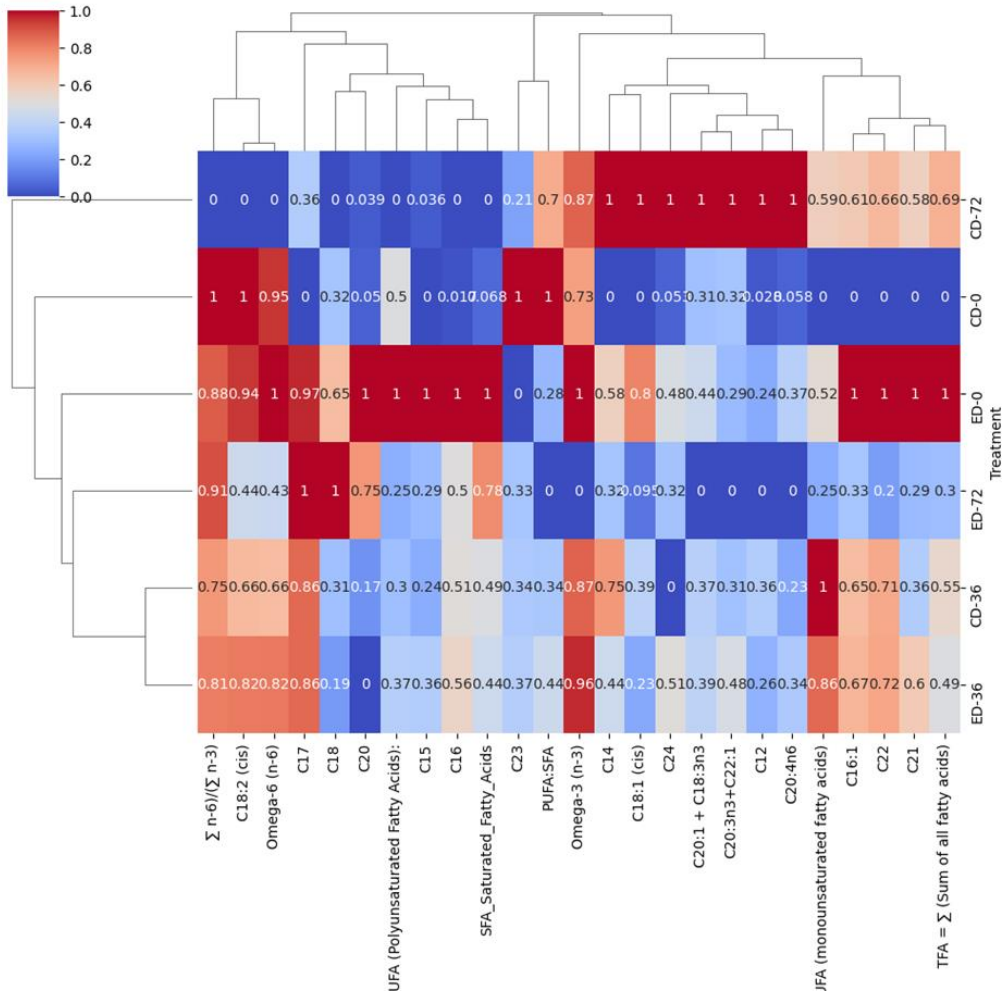
In the 'Beaumont' cultivar, at the end of the storage period, there was a significant positive correlation (0.78) between SFAs and ED-72, while a negative correlation (0.25) was observed between SFAs and PUFAs. As a result, there was a negative correlation (0) between ED-72 and the PUFA:SFA ratio. This negative correlation indicates that the application of

ethylene treatment resulted in a reduction in the ratio of polyunsaturated fatty acids to saturated fatty acids. This decrease can be attributed to the increase in SFA levels, which, in turn, led to a decrease in the overall proportion of polyunsaturated fatty acids. Polyunsaturated fatty acids, namely eicosatrienoic acid + alpha-linolenic, eicosatrienoic acid + erucic acid, and arachidonic acid, were strongly correlated (1) with CD-72. Oleic acid, one of the most abundant MUFAs in macadamia nuts [4,18], was also strongly correlated (1) with CD-72, while SFAs, namely myristic acid, lauric acid, and tetracosanoic acid, were strongly correlated (1) with CD-72 and stearic acid (18:0), heptadecanoic acid (C17:0), and arachidic acid (C20:0) exhibited a strong positive correlation of 1 with ED-72 (Figure 3.4). Moreover, the  $(\sum n-6)/(\sum n-3)$  was strongly correlated (0.91) with ED-72; this is similar to the observation from PCA analysis. In the '788' cultivar (Figure 3.5), at the outset (CD-0), there was a strong positive correlation (correlation coefficient of 1) between total SFAs and the control group (CD), suggesting that the SFA content is high in the control group right after harvesting. In contrast, the correlation with the ethephon-treated group (ED) was negative (correlation coefficient of 0), indicating that the SFA content was initially lower in the ED group. As time progressed to day 36, the correlation between total SFA and both ED and CD increased. For ED, the correlation rose significantly to 0.93, indicating that the SFA content in the ethephon-treated group becomes more aligned with the control group. Similarly, for CD, the correlation also increased to 0.64, suggesting that the SFA content in the control group undergoes some changes. Finally, at the end of the storage period, the correlation remained high for both ED and CD, with ED having a correlation coefficient of 0.86 and CD having a correlation coefficient of 0.73. This suggests that, over time, the SFA content in both groups converges. The rise in the total SFA content in CD-72 could potentially be explained by the fact that lauric acid (with a correlation coefficient of 1), myristic acid (also with a correlation coefficient of 1), heptadecanoic acid (with a correlation coefficient of 0.91), and arachidic

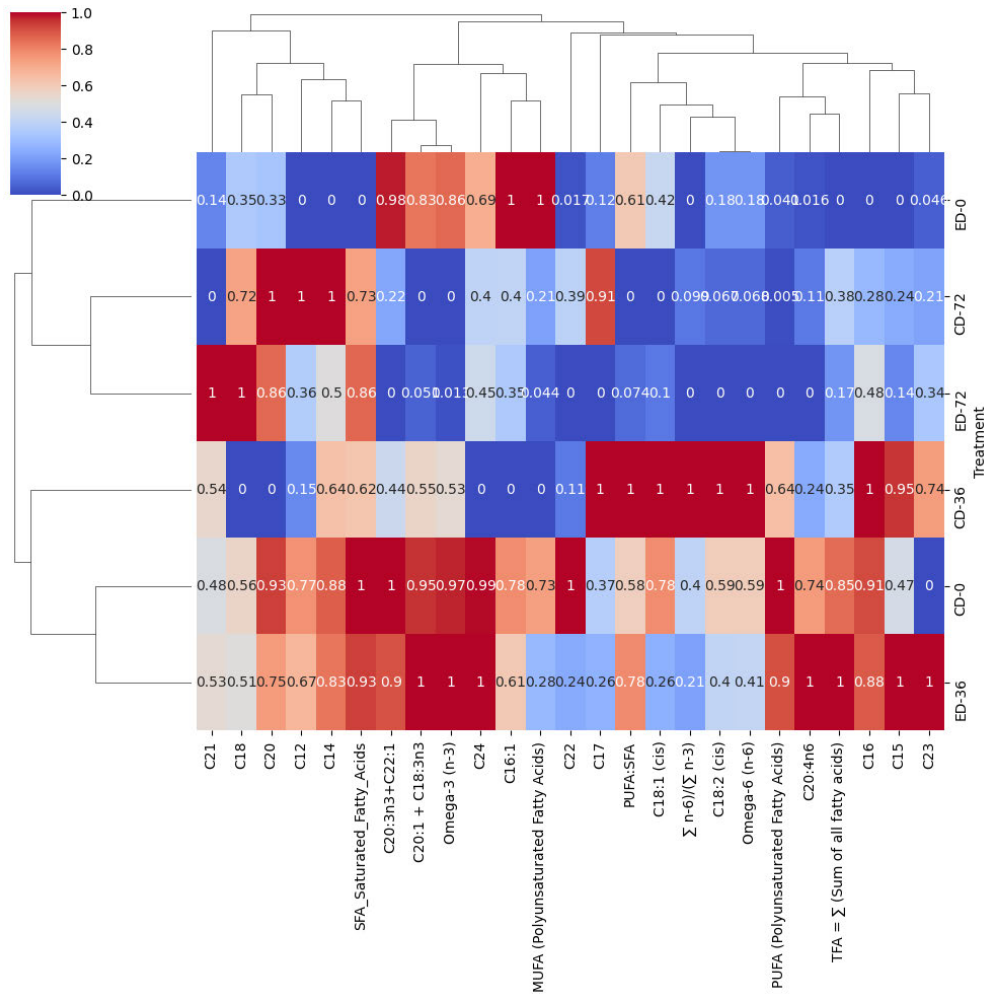
acid (again with a correlation coefficient of 1) exhibited strong positive correlations with CD-72. Furthermore, the increase in SFA content in ED-72 can be attributed to the positive correlations observed with specific saturated fatty acids, including stearic acid (18:0) (correlation coefficient of 1), arachidic acid (C20:0) (correlation coefficient of 0.86), and heneicosanoic acid (C21:0) (correlation coefficient of 1). These SFAs exhibited a strong positive association with ED-72.

At day 0, there was a negative correlation of 0.40 between PUFAs and ED, indicating that the initial PUFA content is lower in the ethephon-treated group compared to the control group (CD). This suggests that ethephon treatment may have initially affected the PUFA levels in the macadamia nuts. In contrast, PUFAs were positively correlated (correlation coefficient of 1) with CD, implying that the control group had a higher PUFA content at the beginning of the study. As the storage period progressed to day 36, PUFAs became strongly correlated with both ED (correlation coefficient of 0.90) and CD (correlation coefficient of 0.64). This suggests that over time, the PUFA content in both groups becomes more similar, possibly due to changes in storage conditions or natural ripening processes. At day 72, PUFAs showed no correlation (correlation coefficient of 0) with either CD or ED. Regarding the PUFA:SFA ratio, at day 0, it had a correlation of 0.61 with ED and 0.58 with CD. This suggests that the initial PUFA:SFA ratios are similar between the two groups. However, by day 36, the PUFA:SFA ratio was strongly correlated with ED (correlation coefficient of 0.78) and highly correlated with CD (correlation coefficient of 1). This indicates that both groups see an increase in PUFA:SFA ratios over the storage period, with CD showing a particularly pronounced change. At day 72, the PUFA:SFA ratio was negatively correlated with both CD and ED. However, it is worth noting that the impact of ethylene treatment on fatty acid composition varied between the 'Beaumont' and '788' cultivars. This indicates that the

response to ethylene treatment might differ depending on the specific cultivar and the stage of storage [34].



**Figure 3.4** Hierarchical clustering heat map of fatty acids in the ‘Beaumont’ cultivar during postharvest storage.



**Figure 3.5** Hierarchical clustering heat map of fatty acids in the ‘788’ cultivar during postharvest storage.

### 3.4.4 Dietary Indices

Table 3.3 illustrates the impact of ethephon on the dietary indices of the ‘Beaumont’ and ‘788’ cultivars during postharvest storage. The atherogenic index (AI), thrombogenic index (TI), and hypocholesterolemic/hypercholesterolemic ratio (h/H) are employed as predictors of cardiovascular risk and improved nutritional value. Lower AI and TI values, along with a high h/H ratio, are considered preferable for this purpose [35].

**Table 3.3** Highlights dietary indices of two macadamia cultivars ('Beaumont' and '788') throughout postharvest storage.

'Beaumont'	Treatment	Day	Atherogenic Indices	Thrombogenic Index	Saturation Index	Lipid Nutritional Value	Hypocholesterolemia/Hypercholesterolemia Ratio
	CD	0	0.1539 ± 0 ef	0.3346 ± 0 ef	0.1648 ± 0 fgh	0.1685 ± 0bc	7.569 ± 0.08 b
	ED	0	0.1553 ± 0 fg	0.3331 ± 0 ef	0.1638 ± 0 efgh	0.1852 ± 0f	7.525 ± 0.02 b
	CD	36	0.1578 ± 0 g	0.3268 ± 0 ed	0.1612 ± 0 def	0.1800 ± 0ef	7.564 ± 0.01 b
	ED	36	0.1570 ± 0 fg	0.3268 ± 0 ed	0.1613 ± 0 defg	0.1834 ± 0ef	7.477 ± 0.01 b
	CD	72	0.1425 ± 0 b	0.2824 ± 0 a	0.1398 ± 0a	0.1549 ± 0.01a	8.61 ± 0.13 e
	ED	72	0.1634 ± 0 h	0.3687 ± 0 g	0.1808 ± 0i	0.1850 ± 0f	7.162 ± 0.04 a
'788'	Treatment	Day	Atherogenic Indices	Thrombogenic Index	Saturation Index	Lipid Nutritional Value	Hypocholesterolemia/Hypercholesterolemia Ratio
	CD	0	0.1506 ± 0 de	0.3263 ± 0.01 de	0.1604 ± 0de	0.1733 ± 0 cd	7.948 ± 0.06 c
	ED	0	0.1328 ± 0 a	0.3027 ± 0.01 b	0.1492 ± 0b	0.1501 ± 0.01 a	8.748 ± 0.12 e
	CD	36	0.1558 ± 0 fg	0.3228 ± 0 cd	0.1594 ± 0d	0.1783 ± 0 de	7.571 ± 0.01 b
	ED	36	0.1461 ± 0 c	0.3158 ± 0 c	0.1555 ± 0c	0.1703 ± 0 bc	8.185 ± 0.03 d
	CD	72	0.1484 ± 0 cd	0.3375 ± 0.01 f	0.1654 ± 0 gh	0.1665 ± 0 b	8.292 ± 0.06 d
	ED	72	0.1493 ± 0 cd	0.3399 ± f	0.1664 ± 0 h	0.1501 ± 0 a	8.748 ± 0.02 e

CD (control drop) and ED (ethephon drop). Values are the mean ± SE. Lowercase letters (a–i) indicate differences between treatments during storage.

In the case of the 'Beaumont' cultivar, the application of ED led to an increase in atherogenic indices, the thrombogenic index, the saturation index, and lipid nutritional value. However, it also resulted in a reduction in the hypocholesterolemia/hypercholesterolemia ratio to 7.16, as opposed to the CD group, which exhibited a ratio of 8.61 on day 72. Elevated atherogenic index (AI) and thrombogenic index (TI) values are implicated in atheroma development and the promotion of platelet aggregation within the cardiovascular system [36]. The thrombogenicity index signifies a predisposition to blood clot formation and has been closely linked to stearic acid [37]. Stearic acid is generally believed to have a neutral impact in terms of atherogenicity. However, it is now being considered as having thrombogenic properties

instead [38,39,40,41]. Therefore, the increase in the thrombogenic index might arise from the significant elevation in stearic acid observed in the ‘Beaumont’ cultivar under ED conditions as well as the strong correlation observed between ED and SFAs. Maintaining lower stearic acid levels holds advantages in preventing cardiovascular disorders. Consequently, the application of ethephon to the ‘Beaumont’ cultivar might yield unfavourable outcomes. Hence, prioritizing lower values is deemed desirable. Regarding the ‘788’ cultivar, at the culmination of the storage period, notable differences in atherogenic indices or the thrombogenic index between the CD and ED groups were not observed. Nevertheless, there was an elevated hypocholesterolemic/hypercholesterolemic ratio in the ED group, indicating a distinct cultivar-specific response to ethephon. The aforementioned results hold implications for nutrition security, a facet aimed at tackling hidden hunger as part of Sustainable Development Goal (SDG) number 2: zero hunger. Nuts play a pivotal role in ensuring nutrition security and are generally favoured by the public for their nutritional benefits. However, the observed increase in stearic acid levels, atherogenic indices, and the thrombogenic index, coupled with a reduction in the hypocholesterolemic/hypercholesterolemic ratio within the ‘Beaumont’ cultivar treated with ethephon, indeed presents a detrimental impact on its nutritional value.

### **3.5 Conclusions**

The present study provides valuable insight into the influence of ethephon on the fatty acid composition and dietary indicators of ‘Beaumont’ and ‘788’ macadamia nut cultivars that abscised due to treatment and without treatment during their postharvest storage period. Our findings reveal significant changes in some fatty acid profiles, particularly in ‘Beaumont,’ where an increase in saturated fatty acids (SFAs) and a decrease in unsaturated fatty acids were observed. This could adversely impact the nutritional quality of the nuts. However, to

comprehensively evaluate the effect of ethephon treatment in light of the nutritional implications, future studies should incorporate sensory evaluations. Additionally, for the ‘Beaumont’ cultivar, ethephon application resulted in an increase in atherogenic and thrombogenic indices, saturation index, and lipid nutritional value while concurrently decreasing the hypocholesterolemic/hypercholesterolemic ratio. These changes, particularly the elevated thrombogenic index, could potentially pose cardiovascular risks. On the other hand, the ‘788’ cultivar produced no significant differences in atherogenic or thrombogenic indices but an increased hypocholesterolemic/hypercholesterolemic ratio, indicating a cultivar-specific effect. Thus, this study hints at the potential for refining strategies to optimize the nutritional value and shelf-life of macadamia nuts, which can be achieved by modifying ethephon treatment methods and carefully selecting cultivars. Specifically, by adjusting ethephon concentrations, application timings, and methodologies, producers can minimize adverse effects on fatty acid composition while maintaining desired ripening effects in macadamia nuts. Additionally, the selection of macadamia cultivars less sensitive to changes in fatty acid profiles can help preserve a more desirable composition in the final product.

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**CHAPTER 4: EFFECT OF PREHARVEST ETHEPHON APPLICATION ON  
SELECTED BIOCHEMICAL COMPONENTS AND POLYPHENOL  
OXIDASE ACTIVITY IN MACADAMIA NUTS**

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## 4.1 Abstract

Ethephon is a plant growth regulator that triggers diverse responses in plants, such as fruit ripening, leaf senescence, hull senescence, stem elongation, and nut abscission. This study examined how the preharvest application of ethephon 480 SL® affects selected biochemical components and polyphenol oxidase (PPO) activity in two macadamia nut cultivars: ‘788’ and ‘Beaumont’. Ethephon was applied to the trees via a Cima mist blower at rates of 13.33 mL/L per hectare for the ‘788’ cultivar and 16.67 mL/L per hectare for the ‘Beaumont’. Following harvest, the nuts were stored at 25 °C for 72 days, and samples were taken at 18-day intervals. Standard procedures were used to assess the following: total phenolics, total flavonoids, 2,2, -diphenyl-1-picrylhydrazyl (DPPH) assay, Ferric reducing ability power (FRAP) assay, sucrose, total protein, and PPO activities. This evaluation was carried out across a total of four treatments: ethephon-treated nuts from the orchard floor (ED), ethephon-treated nuts from the tree (ET), untreated nuts from the orchard floor (CD), and untreated nuts from the tree (CT). The evaluation’s outcomes were analyzed using a principal component analysis (PCA), a correlation matrix heat map (CMHM), and a graphical assessment. The results unveiled significant correlations and associations among the assessed parameters. The correlation matrix heat map analysis highlighted a strong positive correlation (0.97) between the sucrose and the PPO activity in the ‘Beaumont’ cultivar, supported by the PCA analysis identifying the ED treatment as the most influential. At the storage period’s conclusion, the ED treatment had the highest sucrose content (18.63 mg/g) and polyphenol oxidase activity (1.06 U g<sup>-1</sup>L). In the ‘788’ cultivar, a close relationship emerged between the phenolic content, the PPO activity, and the Ferric reducing antioxidant power (FRAP) antioxidant activity. Consistently, the CT treatment (untreated nuts) demonstrated positive correlations with several key parameters in both cultivars, displaying heightened phenolic content and antioxidant activities. Consequently, our findings indicate that the CT treatment,

involving tree-harvested nuts without ethephon application, could be the preferred option for sustaining macadamia nuts' quality and shelf life compared to other methods. Moreover, our study highlights the significance of proper storage conditions for maintaining the desired biochemical parameters of macadamia nuts. By comprehending the effects of distinct treatments and harvesting techniques, producers and processors can devise strategies to optimize storage conditions and uphold macadamia nut quality.

**Keywords:** *ethephon; preharvest; abscission; macadamia nuts; postharvest; quality; nutrition*

## 4.2 Introduction

The cultivation of *Macadamia integrifolia* nuts has gained immense global popularity due to their unique taste, flavour, and nutritional value [1–4]. Macadamia nuts are also well sought-after due to their positive health attributes, with the nuts containing an average of 8% protein and up to eight essential amino acids [3,5,6]. Therefore, macadamia nuts serve as an alternative source of these essential building blocks for optimal health. Furthermore, macadamia nuts are naturally rich in fats, which comprise up to 75% of their total composition [7,8]. Among these fats, approximately 59% are in the form of monounsaturated fat [7]. Nuts are also a source of vitamin B6 and minerals like manganese, iron, and magnesium [9]. Additionally, they contain phytochemicals such as flavonoids and phenolics that further enhance their nutritional profile [10].

Despite these remarkable attributes, the manual harvesting of macadamia nuts is a costly endeavour, requiring significant investments in terms of labour, time, and resources [11]. Therefore, to mitigate such challenges, growers often employ ethephon. Ethephon has been utilized in numerous studies, demonstrating its capacity to induce various plant responses, including fruit ripening, leaf aging, hull aging, stem elongation, and nut detachment [12,13].

When sprayed on macadamia trees, ethephon stimulates the abscission of mature nuts, thus promoting a uniform nut drop. As a result, the cost of multiple harvest periods and the labour required during the harvesting are reduced [11]. The detachment or shedding of plant organs is regulated through a sequence of complex structural, physiological, biochemical, and molecular changes [14]. These changes directly influence the biochemical composition of the organs, including the nuts. Ethephon decomposes into ethylene, a compound recognized for its substantial involvement in various physiological functions within plants [12]. Notably, ethylene has been reported to trigger the expression of plant defence-related proteins [15]. Wang et al. [16] reported that ethephon application in cotton plants resulted in the acceleration of protein degradation in the boll shell and the transfer of assimilated substances [16]. This suggests that ethephon can potentially influence protein degradation and substance transfer in plant structures. Understanding this relationship is essential for obtaining insights into the effects of ethylene on the ripening processes and the resulting changes in biochemical composition. Consequently, the use of ethephon increases the level of ethylene in plant cells, thereby regulating various physiological functions, and can potentially trigger a wide range of physiological mechanisms [17]. The specific effects of this plant growth regulator on the biochemical components and enzymatic activities, such as polyphenol oxidase, of macadamia nuts remain poorly understood. Addressing these knowledge gaps is essential for advancing our understanding of the impact of ethephon on the biochemical composition and polyphenol oxidase activity of macadamia nuts.

Ethephon has been previously used to induce the abscission of various other crops such as peach, table grapes, and litchi [18–20]. Research findings showed that foliar spray of ethephon increases sugar accumulation in sugarcane [21]. Further research conducted by [22] has confirmed that the application of ethylene promotes increased activity in nutrient sinks,

resulting in enhanced sucrose accumulation in genotypes naturally low in sugar content. This finding suggests the potential for cultivars to exhibit different responses to ethephon. Additionally, ref. [23] reported that the application of ethephon led to a reduction in the total anthocyanin, total phenol, soluble solids content, and antioxidant capacity when compared to the control group. These findings imply that ethephon application may have an influence on the biochemical composition of macadamia nuts, particularly regarding their sugar content. Despite the widespread use of ethephon by macadamia growers and the extensive research conducted on the topic [24–27], there remains a notable gap in comprehensive research regarding the postharvest impact of ethephon on the biochemical composition and polyphenol oxidase activity of macadamia nuts. Therefore, the objective of this study is to assess the effect of preharvest foliar spray of ethephon on the postharvest, specifically on the total phenolic content, total flavonoid content, antioxidant capacities (Ferric reducing antioxidant power, and 2,2-diphenyl-1-picrylhydrazyl) and polyphenol oxidase of two distinct macadamia nut cultivars during the storage phase. Additionally, this study aims to evaluate the variation in the biochemical composition and polyphenol oxidase activity between the nuts harvested directly from the tree and those collected from the ground. By addressing these research gaps, this study seeks to provide valuable insights into the postharvest effects of ethephon on macadamia nut quality and shed light on the differences between tree-harvested and ground-harvested nuts.

## **4.3 Materials and Methods**

### ***4.3.1 Ethephon Spray and Concentration***

Macadamia nut cultivars ‘Beaumont’ and ‘788’ were harvested from the Fyvie estates, on uMlaas Road, Camperdown, KwaZulu-Natal, South Africa (latitude: 29°47′50.3″ S.;

longitude: 30°27'54" E.). Ethephon 480 SL®, a plant growth regulator, was used to promote nut abscission in the macadamia trees [28]. The '788' macadamia trees were treated with a dosage of 13.33 mL/L per hectare, while the 'Beaumont' macadamia trees received a dosage of 16.67 mL/L per hectare, both administered using a Cima mist blower, to promote nut abscission. The application of ethephon targeted physiologically mature nuts and the nuts that naturally detached and fell to the ground were considered successfully abscised nuts.

#### ***4.3.2 Nut Collection and Preparation***

The study involved collecting nuts from two distinct cultivars: the 'Beaumont' and the '788'. For each cultivar, the harvested nuts included those fallen from both the treated and untreated trees, as well as those directly picked from both the treated and untreated trees. After collection, a sorting process was used to categorize the nuts into four groups based on their treatment: (CD) for the nuts that had naturally fallen from the control trees; (CT) for the nuts that had been manually picked from the control trees; (ED) for the nuts that had naturally fallen from the ethephon-treated trees; and (ET) for the nuts that had been manually picked from the ethephon-treated trees. Following the harvesting, nuts in their husks underwent mechanical dehusking within 24 h. Subsequently, the dehusked nuts were weighed and subjected to a controlled drying process. The drying procedure followed a specific temperature regimen: 35 °C on the first day, 38 °C on the second day, and 50 °C on the third day. This temperature sequence was adopted in alignment with [29], with minor modifications incorporated to enhance kernel recovery and overall kernel quality.

#### ***4.3.3 Storage Conditions***

In this research study, a total of 600 macadamia kernels were utilized, with 300 kernels assigned to each of the two cultivars, namely the '788' and the 'Beaumont'. Within

each cultivar, these kernels were further divided into four groups: 75 kernels from ET, 75 kernels from ED, 75 kernels from CT, and 75 kernels from CD. These kernels were then organized into three replicates, each comprising five kernels, and securely enclosed in polythene bags. The bags containing the kernels were stored at a temperature of 25 °C for a maximum of 72 days, simulating the conditions that accelerate shelf-life studies. Sampling events occurred at specific intervals of 0, 36, 54, and 72 days to monitor biochemical changes during storage. Following each sampling event, the macadamia nuts were preserved at -20 °C to maintain the quality of the samples for subsequent analysis.

#### ***4.3.4 Extraction and Determination of Total Polyphenols Content and Flavonoids***

The freeze-dried nuts were blended into powder and the extraction was carried out using a method described by [30] with slight modifications. Briefly, 1 g of each sample was suspended in 10 mL of 80% methanol and continuously shaken for 2 h. Thereafter, the samples were filtered through a Whatman® no. 1 and were then vacuum evaporated. After evaporation, the extracts were re-suspended in 2 mL of 80% methanol and finally passed through a 0.45 µm nylon filter to clear all the remaining particles. The extract was assayed for its antioxidant activity, total phenolic content (TPC), and total flavonoid content (TFC).

##### ***4.3.4.1 Quantification of Total Phenolic Content***

The total phenolic content was analyzed as described by [31] with modifications. A sample extract of 100 µL was mixed with 0.2 mL of Folin–Ciocalteu reagent and 1 mL of distilled water, and the solution was allowed to shake for 3 min at room temperature. Thereafter, 1 mL of 20% sodium carbonate was added to the mixture. The total phenolic content was quantified after 90 min of incubation at room temperature (25 °C). The absorbance of the solution was measured at 765 nm using a Shimadzu UV-VIS spectrophotometer. A standard curve of gallic acid was used for the TPC's quantification. The

results were expressed as mg of gallic acid equivalents (GAE) per g of dry weight (DW). The analysis was conducted in triplicate.

#### ***4.3.4.2 Quantification of Total Flavonoid Content***

The total flavonoids were quantified following a method described by [32] using Aluminum Chloride (AlCl<sub>3</sub>). A sample extract of 100 µL was added into a cuvette followed by an addition of 200 µL of water followed by 30 µL of 5% NaNO<sub>2</sub>, and the mixture was allowed to stand at room temperature (25 °C) for 5 min. Subsequently, 30 µL of 10% AlCl<sub>3</sub> was added; the mixture was incubated for 6 min, and, thereafter, 200 µL of 1 mM NaOH was added to the solution. The absorbance of the reaction was read at 510 nm against methanol used as a blank. A standard curve of quercetin was used for the TFC's quantification. The results were expressed as mg of quercetin equivalents per g of dry weight (DW). The analysis was conducted in triplicate.

#### ***4.3.5. 2,2,-Diphenyl-1-picrylhydrazyl (DPPH) Assay***

The free radical scavenging activity using the DPPH assay was determined following the method described by [33] with slight modifications. A stock solution of 2,2,-diphenyl-1-picrylhydrazyl at a concentration of 0.1 mM was prepared using methanol and was stored at -20 °C until further use. For the assay, 20 µL of the kernel extract was added to a cuvette, followed by the addition of 980 µL of methanol. Subsequently, 1 mL of the DPPH solution was added to the mixture. The solution was then incubated in the dark for 1 h. After incubation, the change in absorbance was measured at 517 nm under dim light using a Shimadzu US-VIS spectrophotometer. The analysis was conducted in triplicate. The inhibition percentage was calculated using Equation (1):

$$DPPH = \frac{Abs\ control - Abs\ sample}{Abs\ control} \quad (1)$$

where Abs<sub>control</sub> is the absorption of the DPPH radical and methanol, and Abs<sub>sample</sub> is the absorption of the DPPH radical and sample extract.

#### **4.3.6. Ferric Reducing Ability Power (FRAP) Assay**

The Ferric reducing ability power (FRAP) assay was determined according to [34]. The FRAP reagent was prepared by mixing 60 mL of acetate buffer (300 mmol/L, pH 3.6), 6 mL ferric chloride solution (20 mmol/L), and 6 mL of 10 mmol/L 2,4,6-tripyridyl-s-triazine (TPTZ) solution (TPTZ in 40 mM HCl) in a ratio of 10:1:1. The mixture was allowed to stand in a water bath at 37 °C for 15 min. The FRAP reagent (600 µL) was added to 80 µL of extract and 1 mL of water. A reagent blank was prepared as above, with 80 µL water added instead of the test sample. The change in absorbance was recorded at 593 nm using a spectrophotometer. The analysis was conducted in triplicate. A standard solution of ferrous sulphate was prepared. The total antioxidant capacity was expressed as µmoles Fe<sup>2+</sup>/g of dry weight (DW).

#### **4.3.7. Sucrose Analysis**

The sugar content was determined using an HPLC-refractive-index detector (RID) based on the method outlined by [35] with slight modifications. Freeze-dried material weighing 0.10 g was mixed with 10 mL of 80% (v/v) ethanol and homogenized for 1 min. The mixture was then incubated in an 80 °C water bath for 60 min to extract the soluble sugars. Following incubation, the mixture was refrigerated at 4 °C overnight. The samples were subsequently filtered through glass wool and dried in a vacuum concentrator. The dried samples were then re-suspended in 2 mL of ultra-pure water, filtered through a 0.45 µm nylon

filter, and analyzed using an isocratic HPLC system equipped with an RID. A Phenomenex® column (Rezex RCM–Monosaccharide) was used for the analysis. The concentration of sucrose was determined by comparing the samples to the known sucrose standard.

#### ***4.3.8. Protein Extraction***

Freeze-dried nuts were ground into a powder, and the extraction was performed using a modified method based on [36]. In this process, 0.5 g of each sample was suspended in 5 mL of TRIS buffer (100 mM, pH 7.5). Subsequently, the samples were centrifuged at 10,000 RPM for 15 min at 4 °C. The supernatant was then filtered through glass wool to remove any remaining floating particles, and the extract was stored for further protein analysis.

##### ***4.3.8.1 Protein Assay Using the Bradford Method***

The protein assay was conducted following the procedure described by [36] with slight modifications. To begin, 400 µL of the Bradford reagent was added to 40 µL of the protein extract. The mixture was incubated for 5 min and then read at an absorbance of 590 nm. The results were expressed as micrograms of bovine serum albumin (BSA) per gram of dry weight (DW). The analysis was performed in triplicates.

##### ***4.3.8.2 Quantification of Polyphenol Oxidase***

The activity of polyphenol oxidase (PPO) was determined following the method described by [29], which is based on the assay previously outlined by [37]. To conduct the assay, a 100 mL sample of extracted protein was mixed with a combination of 1.45 mL of 20 mM 3-methyl-catechol and 1.45 mL of 10 mM acetate buffer (pH 5.0). The total PPO activity was measured using a spectrophotometer at a wavelength of 420 nm and was expressed as U g<sup>-1</sup> of sample dry weight.

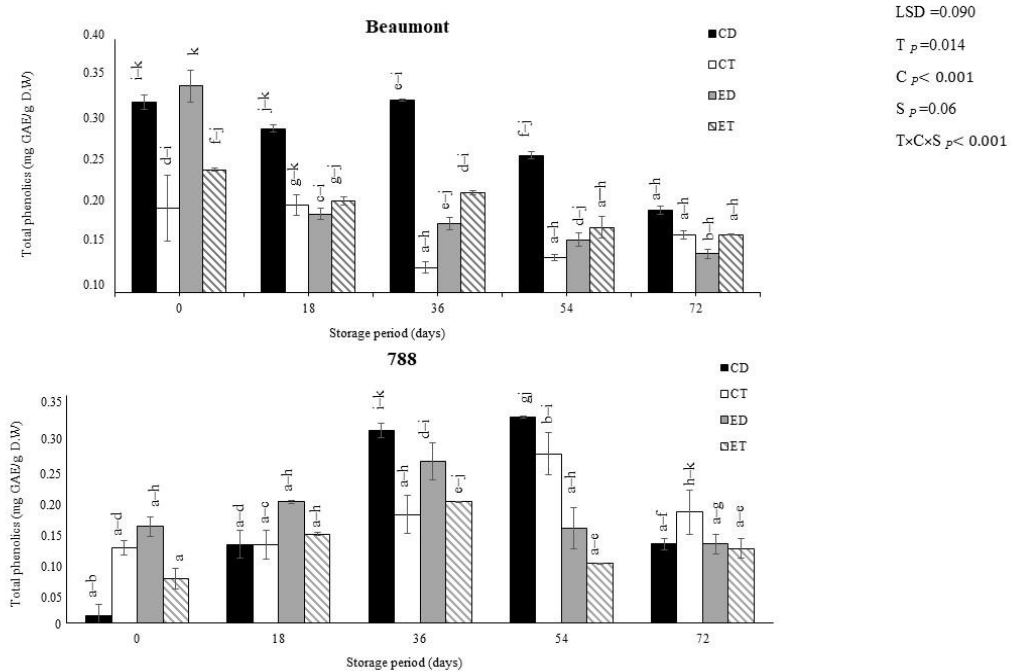
#### **4.3.9. Data Analysis**

An analysis of variance (ANOVA) was carried out using GenStat® 20th Edition (VSN International Ltd., Hertfordshire, UK). The means were separated using the least significant difference (LSD) test at 5% levels of significance. A principal component analysis (PCA), biplot diagrams, and a correlation matrix heatmap were deployed to facilitate the comprehensive identification of the effect of ethephon on macadamia nuts' postharvest quality using Python programming.

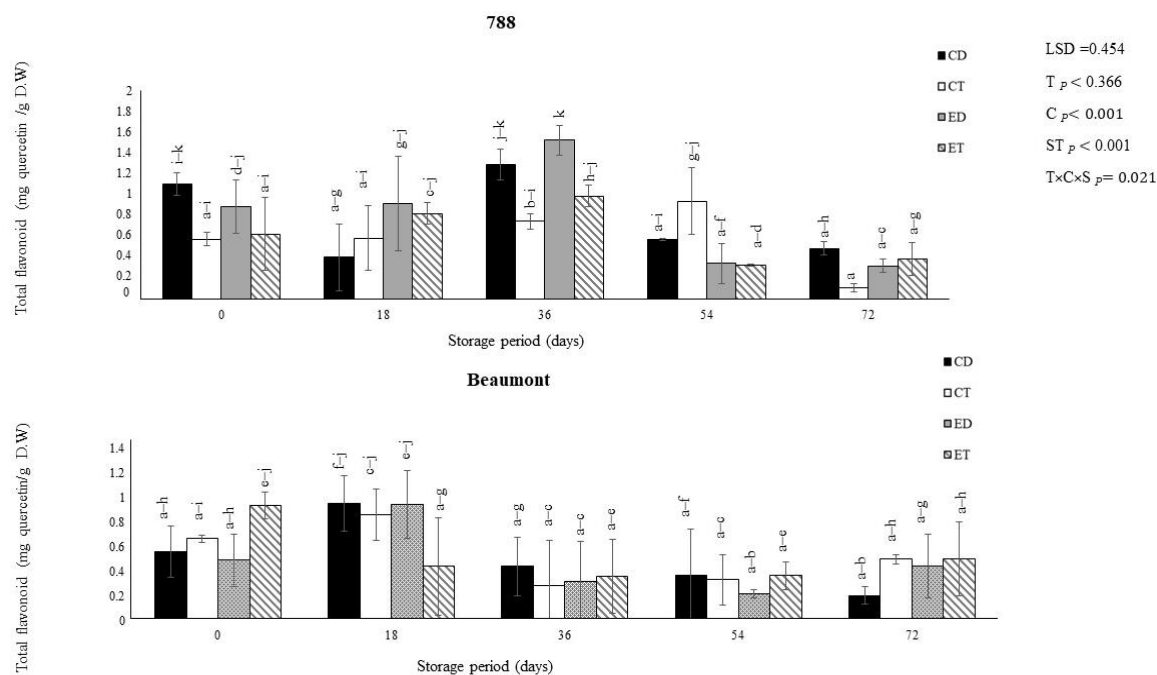
### **4.4 Results and discussion**

#### **4.4.1. Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC)**

Flavonoids and phenolics are polyphenolic compounds that have numerous health benefits and occur naturally in a wide range of foods, including macadamia nuts [3,30]. The impact of different treatments (CD, CT, ED, and ET) on the TPC and TFC of macadamia nuts ('Beaumont' and '788') stored at 25 °C is depicted in Figures 4.1 and 4.2, respectively. For the 'Beaumont' nuts, the ET decreased over time but had the highest phenolic treatment among all treatments (except for the CD) on day 0. The decrease in the TPC in the ET and ED nuts could be attributed to the influence of the ethylene released from the ethephon, which can induce enzymatic degradation of phenolic compounds (Figure 4.5) [38,39]. On days 0 and 18, the CD nuts maintained a higher TPC compared to the ET nuts. This observation suggests that the CD treatment may have promoted the accumulation or preservation of phenolic compounds during the initial stages of storage in the control treatment, resulting in an increased TPC. Conversely, for the '788' nuts, all treatments generally resulted in an increase in TPC up to day 36 and then a decrease. The CT treatment had the highest TPC (0.25 mg GAE/g) at the end of the storage period.



**Figure 4.1** The effect of ethephon on the total phenolic content of macadamia nuts during storage. Data are expressed as mean  $\pm$  standard errors of three replicates. T represents treatment (ethephon or control); C stands for cultivar; and S represents storage duration in days.

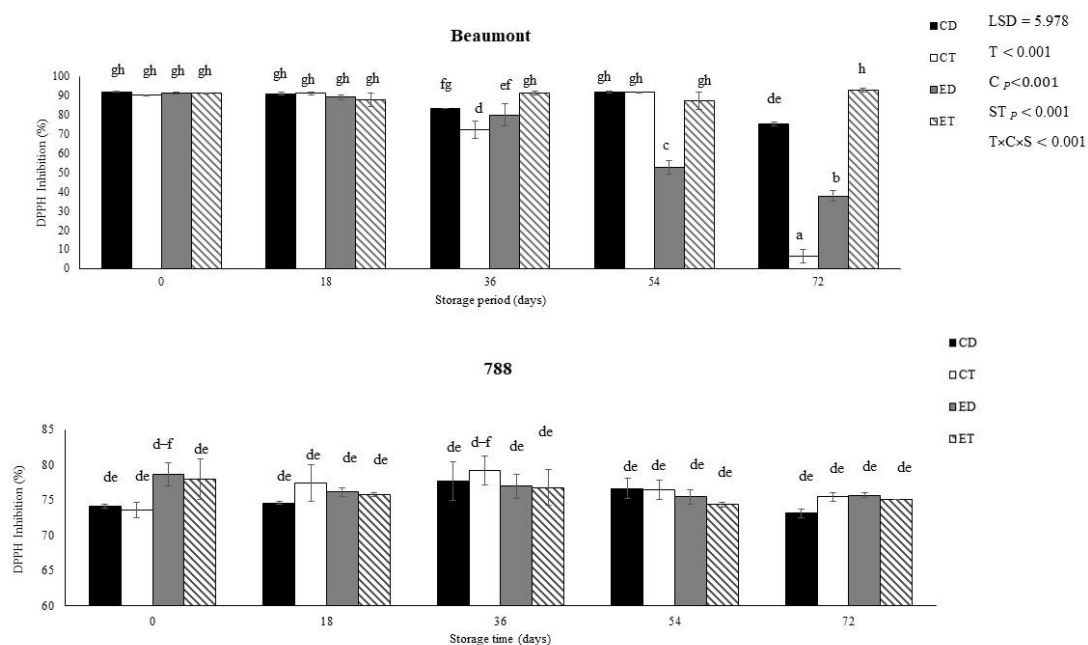


**Figure 4.2** The effect of ethephon on the total flavonoid content of macadamia nuts during storage. Data are expressed as mean  $\pm$  standard errors of three replicates. Vertical bars represent the standard errors of the means. T represents treatment (ethephon or control); C stands for cultivar; and S represents storage duration in days.

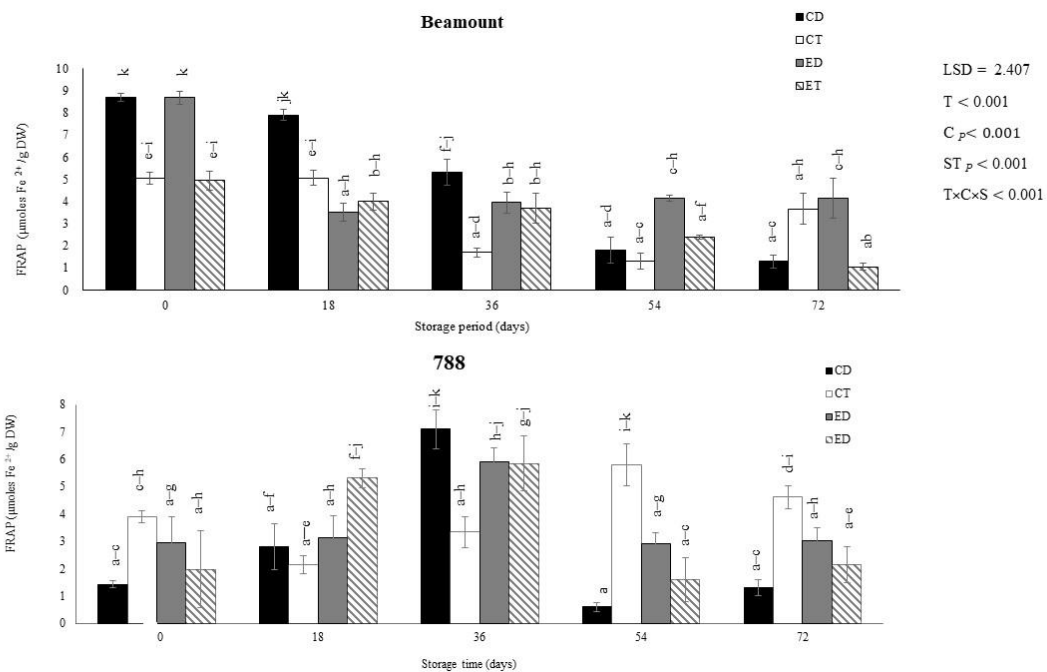
#### 4.4.2. Antioxidant Activity (DPPH and FRAP)

The assessment of the DPPH and FRAP radical scavenging rates is a common method used to evaluate the antioxidant capacity of fruits and vegetables [40]. The impact of different treatments (CD, CT, ED, and ET) on the DPPH and the FRAP of macadamia nuts ('Beaumont' and '788') stored at 25 °C is depicted in Figures 4.3 and 4.4, respectively. For the cultivar 'Beaumont', the DPPH inhibition percentage (%) was generally high for all treatments, indicating a strong antioxidant activity, except for the control drop (CT) and ethephon drop (ED) treatments. The CT and ED exhibited the lowest DPPH inhibition percentage (5.44% and 53.22%, respectively) at the end of the storage period. For the cultivar '788', the DPPH was maintained throughout the whole storage period and there were no

significant differences observed between the treatments. Therefore, the ethephon had no significant effect on the DPPH of the ‘788’ harvested nuts. In the case of the cultivar ‘Beaumont’, the CD and ED displayed the highest FRAP values of 8.69 and 8.68  $\mu$  moles, respectively on day 0 (Figure 4.4). Increases in FRAP have been attributed to high TPC and TFC [41]. Therefore, the higher FRAP observed could be a result of the high TPC and TFC observed. After day 0, the ED decreased while the CD maintained the highest FRAP out of all the treatments. On the other hand, for cultivar ‘788’, the CT treatment exhibited the highest FRAP values on day 54 (7.38  $\mu$ moles). The ethephon treatment increased from day 0 to 36 and then decreased.



**Figure 4.3** The effect of ethephon application on the DPPH of macadamia nuts during storage. Data are expressed as mean  $\pm$  standard errors of three replicates. Vertical bars represent the standard errors of the means. T represents treatment (ethephon or control); C stands for cultivar; and S represents storage duration in days.

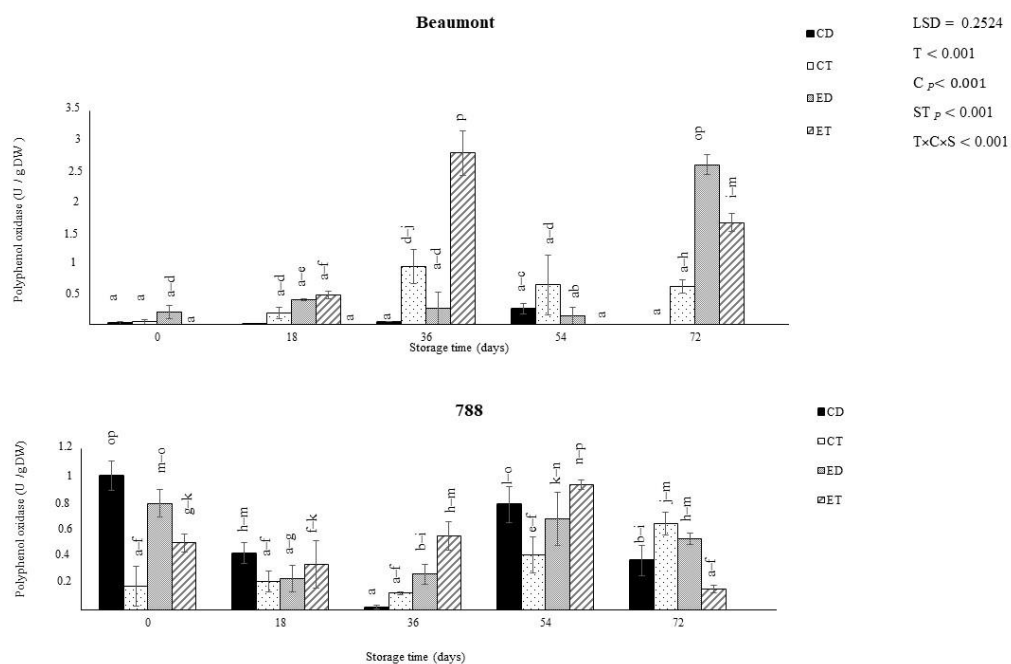


**Figure 4.4** The effect of ethephon application on the FRAP of macadamia nuts during storage. Data are expressed as mean  $\pm$  standard errors of three replicates. T represents treatment (ethephon or control); C stands for cultivar; and S represents storage duration in days.

#### 4.4.3. Polyphenol Oxidase Activity

Polyphenol oxidase (PPO) is an enzyme responsible for the browning of fruits when exposed to the atmosphere or physical abrasions [42]. Figure 4.5 demonstrates the impact of various treatments (CD, CT, ED, and ET) on the PPO's activity in macadamia nuts ('Beaumont' and '788') stored at 25 °C. In the 'Beaumont' cultivar, the control treatments

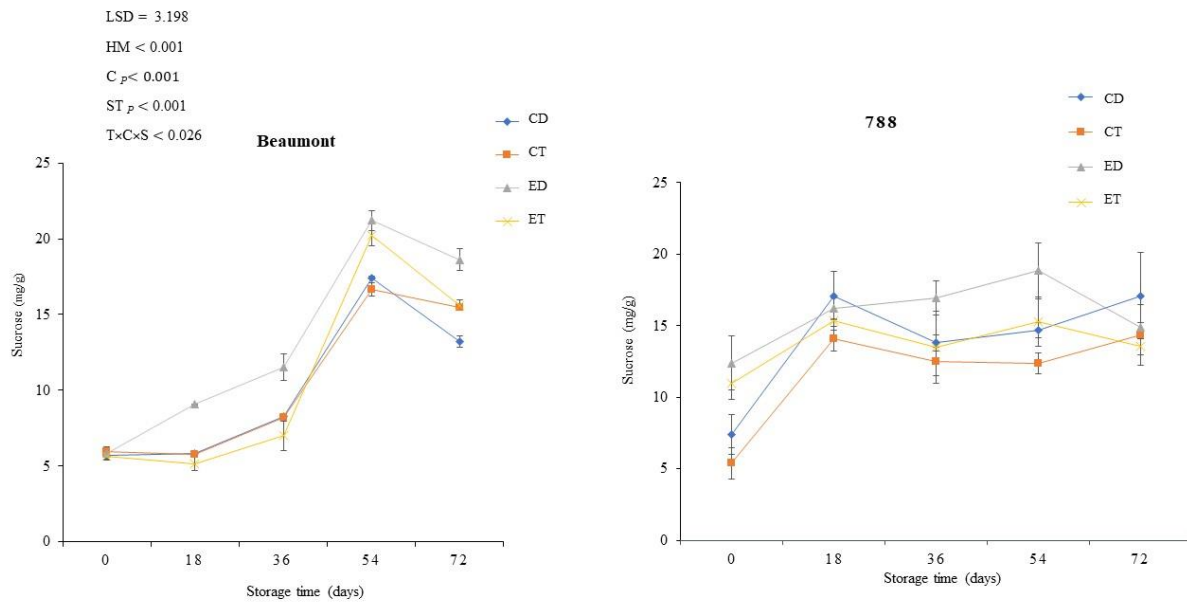
maintained a consistently low PPO activity throughout the storage period, while the ethephon treatments (ED and ET) exhibited the highest PPO activity (1.06 and 0.58 U g<sup>-1</sup>L, respectively) at the end of the storage period. Ethephon, which releases ethylene, accelerates senescence in both climacteric and non-climacteric fruits and vegetables [43]. Our findings align with those of [44], who reported that ethephon treatment increases PPO activity and accelerates senescence. For the ‘788’ cultivar, however, the ET and the ED as well as the CD treatment displayed a high PPO activity from the beginning of the storage period, while the CT treatment initially showed the lowest activity (0.18 U g<sup>-1</sup>) on day 0. This indicates a differential response among the two cultivars with the CD treatment being more prone to PPO activity in the ‘788’ cultivar.



**Figure 4.5** The effect of ethephon application on the polyphenol oxidase activity of macadamia nuts during storage. Data are expressed as mean  $\pm$  standard errors of three replicates. Vertical bars represented the standard errors of the means. T represents treatment (ethephon or control); C stands for cultivar; and S represents storage duration in days.

#### **4.4.4. Sucrose Content**

Sucrose is the predominant sugar in macadamia nuts, although small amounts of reducing sugars have also been reported [45,46]. The impact of various treatments (CD, CT, ED, and ET) on the sucrose content of macadamia nuts ('Beaumont' and '788') stored at 25 °C is illustrated in Figure 4.6. In the case of the cultivar 'Beaumont', the sucrose content increased for all the treatments over the storage period. However, the ED treatment exhibited a more pronounced increase and ultimately had the highest sucrose content (18.63 mg/g) by the end of the storage period. Similarly, in the cultivar '788', the ED treatment displayed a high sucrose (18.84 mg/g) content up to day 54. Additionally, the CT treatment showed a consistently lower sucrose content (5.39–12.35 mg/g) from the beginning of the storage period until day 54. The application of ethephon, a plant growth regulator, can influence the sucrose metabolism in macadamia nuts [47]. Studies have revealed that the presence of sucrose synthase and sucrose-phosphate synthase, which regulate the production of sucrose in plants, can be stimulated through ethephon exposure [47]. This could be the possible reason for the high sucrose content observed in the ethephon-treated nuts for both cultivars. This observation is also similar to the findings of [48], who indicated that sucrose synthase activities were found to be higher in the first 90 days in fruits exposed to ethephon [48]. Also, the variation observed for the different cultivars is similar to the findings of [22], who reported that ethylene treatment increased the stem sucrose content of the low-sugar genotype of sugarcane compared to the high-sugar genotype of sugarcane. The workers further asserted that the sucrose and starch metabolism genes were found to be responsive to ethylene [22].

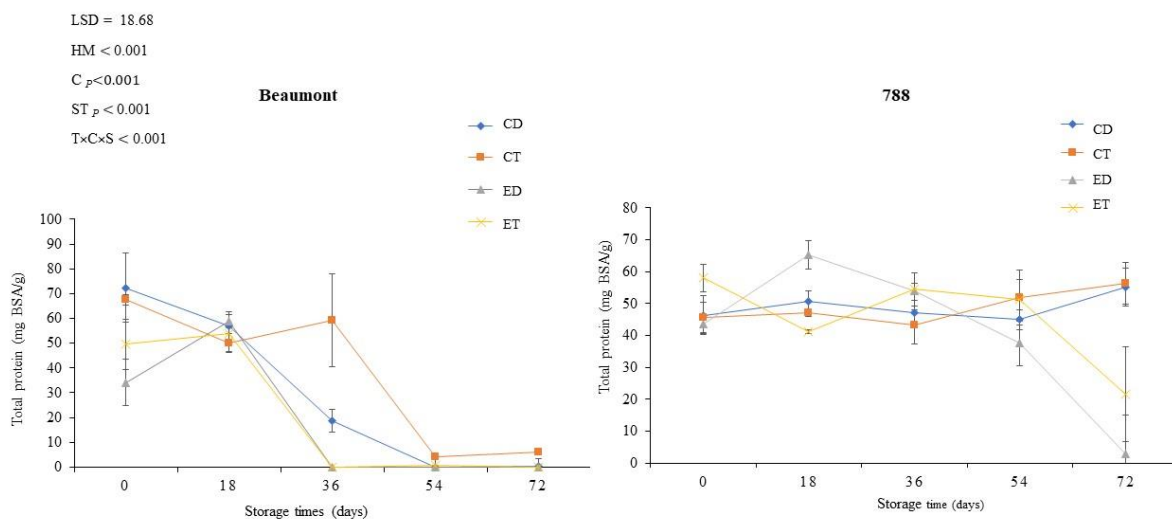


**Figure 4.6** The effect of ethephon application on the sucrose content of macadamia nuts during storage. Data are expressed as mean ± standard errors of three replicates. Vertical bars represent the standard errors of the means. T represents treatment (ethephon or control); C stands for cultivar; and S represents storage duration in days.

**4.4.5. Total Protein Content**

Nuts are a valuable plant-based source of protein, and macadamia nuts typically contain around 8% of protein [49]. The impact of different treatments (CD, CT, ED, and ET) on the total protein content of macadamia nuts (‘Beaumont’ and ‘788’) stored at 25 °C is shown in Figure 4.7. For the ‘Beaumont’ cultivar, the CD and CT treatments initially exhibited a high protein content (72.4 and 67.5 mg BSA/g, respectively); but, over time, the protein content gradually decreased, reaching 0.5 mg BSA/g for the CD and 6.2 mg BSA/g for the CT by day 72. This decline in protein content can be attributed to the denaturation and solubilization processes that occur at elevated temperatures [50]. Similarly, in the ‘788’

cultivar, the CT and CD treatments showed the highest total protein content (55.2 and 56.4 mg BSA/g, respectively) at the end of the storage period, while the ET and ED treatments had lower protein contents (21.6 mg BSA/g for ET and 2.9 mg BSA/g for ED). In the ‘Beaumont’ cultivar, all the treatments led to a general decrease in the protein content, but the reduction was more pronounced in the ET and ED nuts for both cultivars. This significant decrease in protein content could be attributed to the induced degradation of specific proteins, such as ethylene receptor 2 (ETR2), which occurs at the mRNA (post-transcriptional) level through a proteasome-dependent pathway [51].

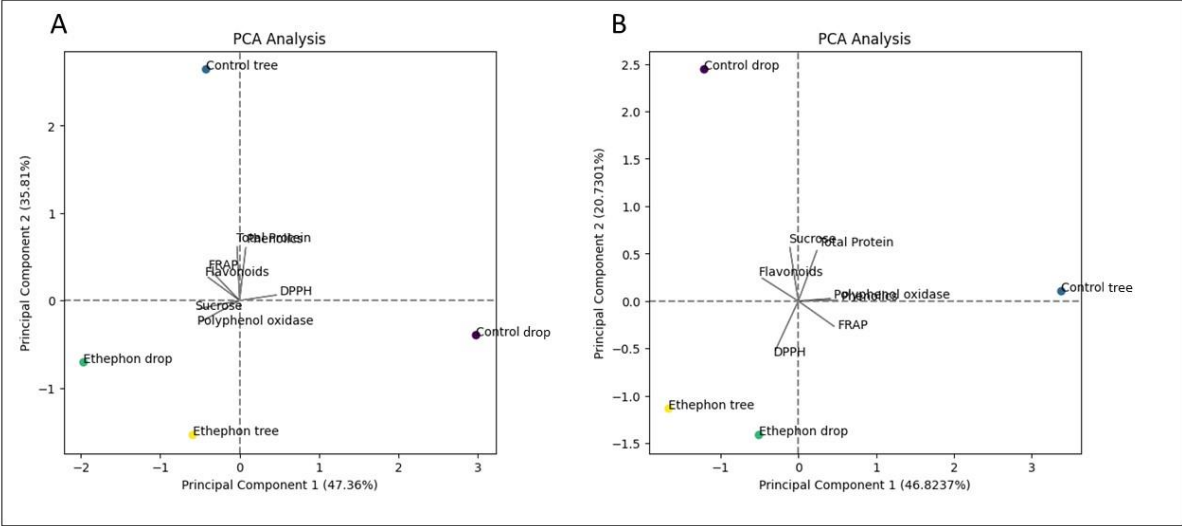


**Figure 4.7** The effect of ethephon application on the total protein content of macadamia nuts during storage. Data are expressed as mean ± standard errors of three replicates. Vertical bars represent the standard errors of the means.

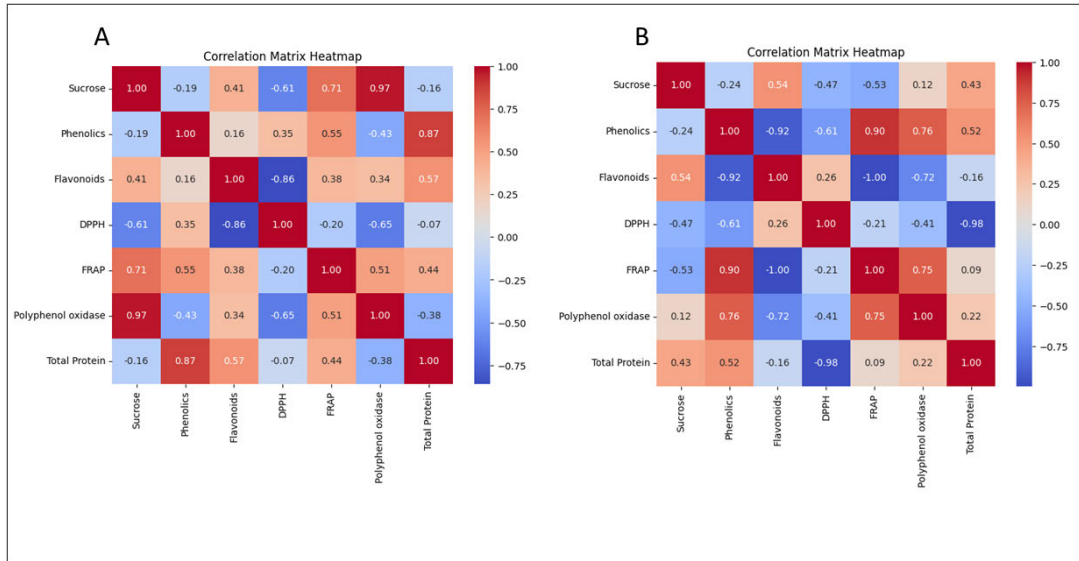
#### ***4.4.6. Correlation Matrix Heatmap and Principal Component Analysis (PCA)***

The present study conducted an analysis of the principal components of the evaluated biochemical parameters in the ‘Beaumont’ and ‘788’ cultivars using various visualization techniques, including a PCA biplot (Figure 4.8), a heat map (Figure 4.9), and PCA 3D plots (Figure 4.10). The correlation matrix was employed to measure the linear relationship between the parameters, ranging from -1 to +1, where correlations greater than or equal to 0.75 or less than or equal to -0.75 were considered significant. For the ‘Beaumont’ cultivar, the PCA biplot analysis revealed that PCA 1 explained 47.36% of the total variation, while PCA 2 accounted for 35.81% of the variation. Notably, the vectors representing sucrose and PPO were closely aligned in the biplot, indicating a strong positive correlation between these two biochemical parameters. This finding was further supported by the heat map, which displayed a correlation coefficient of 0.97 (Figure 4.9A). This observation is consistent with the findings of [52], who studied the PPO activity in in vitro rejuvenated and mature shoots of birch. They reported that a high sucrose content was associated with an increased PPO activity. This could be attributed to the sucrose-enhancing phenolic synthesis, which in turn promotes the activity of PPO [52]. Additionally, the directions of the vectors for sucrose and polyphenol oxidase pointed towards the ED treatment, implying a positive correlation with this treatment (Figures 4.8A and 4.10A). Similarly, the direction of the total protein vector pointed towards the CT treatment, suggesting a strong correlation with this particular treatment (Figures 4.8A and 4.10A). This observation of close proximity and strong correlation was also observed between the total protein and TPC, with a correlation coefficient of 0.87 (Figures 4.8A and 4.9A). However, it is important to note that, despite the apparent proximity of the vectors representing the FRAP and flavonoids in the PCA biplot, the PCA 3D plot provided a clearer view indicating the non-proximity and lack of correlation between these two parameters (Figures 4.8A and 4.10A). This observation was further

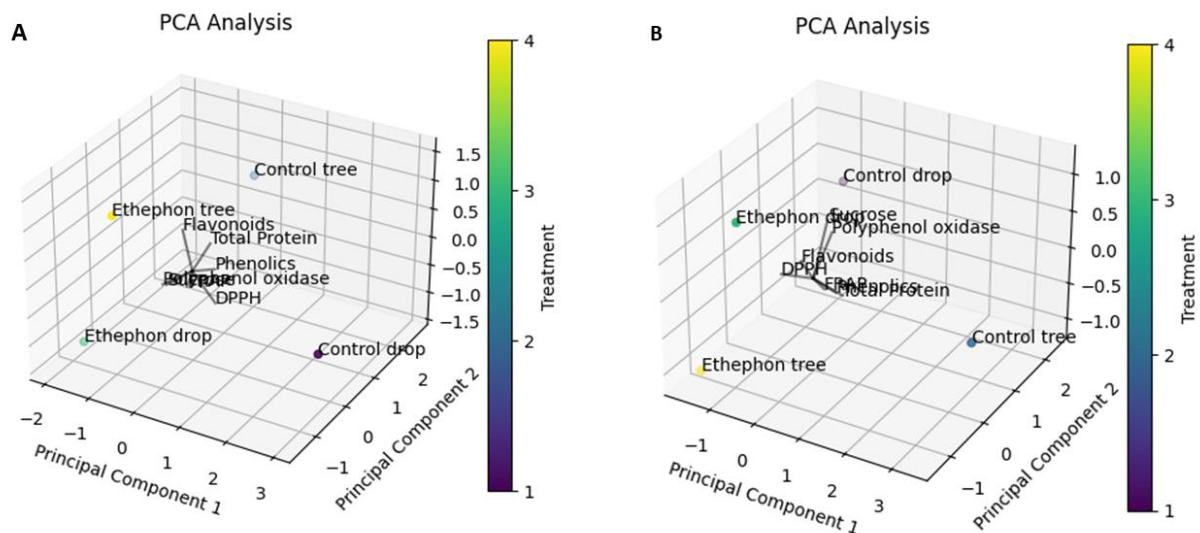
supported by the heat map, which revealed a correlation coefficient of 0.38 (Figure 4.9A). Likewise, a strong negative correlation (-0.87) was observed between the FRAP and DPPH, as confirmed by the heat map (Figure 4.9A).



**Figure 4.8** Principal component (PC) biplot of PC1 vs. PC2 showing the relationship between the quality parameters and the different treatments of two macadamia nuts—(A) ‘Beaumont’ and (B) ‘788’—during storage.



**Figure 4.9** The heatmap representing the correlations between the phenolic contents, flavonoid content, antioxidant activities (FRAP and DPPH), PPO, and total protein during the storage of (A) 'Beaumont' and (B) '788' macadamia nuts. Red and blue colours, respectively, indicate positive and negative correlations; the deeper colours indicate a stronger correlation.



**Figure 4.10** 3D Principal component (PC) biplot of PC1 vs. PC2 showing the relationship

between the quality parameters and the different treatments of two macadamia nuts—(A) ‘Beaumont’ and (B) ‘788’—during storage.

For the ‘788’ cultivar, the analysis of the principal components using the PCA biplot indicated that PCA 1 accounted for 46.82% of the total variation, while PCA 2 explained 20.73% of the variation. Notably, the vectors representing the polyphenol oxidase and TPC were closely aligned in the biplot, suggesting a strong correlation between these two parameters (Figure 4.8B). This observation was further supported by the heat map, which displayed a correlation coefficient of 0.76 for the polyphenol oxidase and TPC parameters (Figure 4.9B). Phenolic compounds are known to serve as substrates for polyphenol oxidase during the oxidative browning process [53]. Enzymatic browning is the undesirable colour change in food caused by the conversion of phenolic compounds that negatively impacts the food’s sensory and nutritional properties [54]. This suggests that the presence of TPC may play a role in activating or influencing the activity of polyphenol oxidase, potentially contributing to the oxidative browning reactions in the macadamia nuts. Furthermore, the vectors of TPC and FRAP were in close proximity to each other, suggesting a strong correlation between these two parameters (Figure 4.8 and Figure 4.10). This finding was consistent with the heat map, which revealed a correlation coefficient of 0.9 for the TPC and FRAP parameters (Figure 4.9). Previous studies have reported a correlation between the TPC and antioxidant activity, as assessed by the FRAP assay [55,56]. Another significant observation was the proximity of the vectors representing the FRAP and polyphenol oxidase (Figure 4.8). This proximity further suggested a strong correlation between these two

parameters, which was supported by the heat map displaying a correlation coefficient of 0.75 (Figure 4.9). The presence and accumulation of phenolic compounds are closely associated with antioxidant capacity [40]. These findings indicate that the activity of polyphenol oxidase may be influenced by the presence of phenolic compounds, as discussed earlier. Additionally, the alignment of vectors representing the TPC, FRAP, and total protein parameters toward the CT treatment suggests a strong correlation between these parameters and this treatment. This implies that the nuts harvested from the CT treatment exhibit higher levels of total phenolic content, FRAP antioxidant activity, and total protein. However, it is crucial to note that it is not advisable to leave nuts hanging on the tree for several months as it can lead to a deterioration in quality [57].

#### **4.5 Conclusions**

Our analysis of the effect of ethephon on the biochemical parameters and polyphenol oxidase (PPO) activity of the ‘Beaumont’ and ‘788’ cultivars of macadamia nuts harvested through different methods (CT, CD, ET, and ED) during postharvest storage, revealed significant correlations and associations among the evaluated parameters. In the ‘Beaumont’ cultivar, we observed a strong positive correlation (0.97) between the sucrose and the polyphenol oxidase. Specifically, the nuts treated with the ethephon and harvested from the orchard floor (ED) exhibited the highest sucrose content (18.63 mg/g) and polyphenol oxidase activity (1.06 U g<sup>-1</sup>L) at the end of the storage period. In the ‘788’ cultivar, our analysis demonstrated a close relationship between the phenolics, the polyphenol oxidase (PPO), and the FRAP antioxidant activity. This suggests that the concentration of phenolic compounds in macadamia nuts is closely associated with the activity of polyphenol oxidase, an enzyme involved in browning reactions, as well as the overall antioxidant capacity measured by FRAP. The strong association between these factors indicates that the phenolics present in the

nuts may contribute to the oxidative browning process and play a role in their antioxidant activity. Considering these findings, the CT treatment emerges as the most favorable option as it consistently showed positive correlations with multiple key parameters in both cultivars. This suggests that nuts harvested using the CT method may have a longer shelf life due to their higher phenolic content and antioxidant activity. However, it is important to acknowledge that the CT treatment, which involves harvesting nuts directly from the tree, can be labor-intensive. In contrast, the ethephon treatment is easier to implement but may not yield the same level of desired outcomes. Therefore, for future considerations, it would be beneficial to focus on optimizing the biochemical parameters and minimizing the polyphenol oxidase (PPO) activity of nuts harvested from ethephon-treated trees in order to improve their quality during storage. Further research efforts can explore strategies to enhance the shelf life and maintain the nutritional quality of nuts treated with ethephon, potentially bridging the gap between the convenience of implementation and the desirable outcomes. Further investigations can explore strategies to enhance the shelf life and preserve the nutritional value of nuts treated with ethephon, considering factors such as storage conditions, postharvest treatments, and processing techniques.

#### 4.6 References

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**CHAPTER 5: INVESTIGATING THE IMPACT OF ETHEPHON ON THE  
PHYSICAL ATTRIBUTES OF ROASTED MACADAMIA NUTS DURING  
ACCELERATED STORAGE**

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## 5.1 Abstract

Macadamia nuts, renowned for their unique flavour and texture, undergo transformative changes during roasting. Despite the widespread application of ethephon as a harvesting tool in macadamia nut production, its influence on the physical attributes and colour changes of roasted nuts remains underexplored. Ethephon 480 SL®, a plant growth regulator, was applied doses based on industry recommendations for each cultivar: ‘788’ trees received 13.33 mL per liter per hectare, and ‘Beaumont’ trees were treated with 16.67 mL per liter per hectare, administered via a Cima mist blower. This study categorizes nuts into four groups based on treatments: naturally fallen nuts from untreated trees (CD), manually picked nuts from untreated trees (CT), naturally fallen nuts from ethephon-treated trees (ED), and manually picked nuts from ethephon-treated trees (ET). Following drying and dehusking nuts were roasted at 125 °C for 15 minutes. Cooled nuts were stored at 25 °C, with samples collected at 0, 14, 28, 42, and 56 days. Comprehensive analyses of physical attributes, including mass, diameter, colour, and texture, were conducted. ED had a distinct impact on the physical attributes of the ‘Beaumont’ cultivar, with notable effects on parameters such as  $a^*$ ,  $b^*$ , mass, and diameter. Conversely, in the ‘788’ cultivar, Principal Component Analysis (PCA) revealed that CD treatment held prominence, showing elevated values for firmness,  $a^*$ , and  $b^*$ . These findings offer practical insights for industry practitioners, emphasizing the importance of considering cultivar-specific responses and the maturity stage during ethephon application. The study not only addresses immediate implications for macadamia cultivation but contributes to the broader understanding of optimal postharvest practices, ultimately enhancing the production of superior macadamia nut products. In conclusion, this research, guided by industry-recommended doses, sheds light on the nuanced effects of ethephon application, providing valuable insights for enhancing macadamia nut quality.

**Keywords:** Macadamia nuts, ethephon, preharvest, postharvest, roasting, shelf life, colour changes, texture.

## 5.2 Introduction

Macadamia nuts are known for their rich flavour and nutritional value. Macadamia nuts are cultivated in regions across the globe, with South Africa and Australia serving as prominent producers (Wessell, 2022; Shabalala et al., 2022). These nuts, primarily belonging to the species *Macadamia integrifolia* and *Macadamia tetraphylla*, are esteemed for their high levels of monounsaturated fats, essential vitamins, and minerals (Buthelezi et al., 2019; Wall, 2013). They are versatile, and commonly enjoyed raw or roasted delicacies (Silva et al., 2006). Roasting enhances macadamia nuts' sensory qualities, making them preferred by consumers due to improved flavour, aroma, texture, and appearance (Tu et al., 2021). The conventional practice is to harvest macadamia nuts upon reaching maturity. However, a long-standing challenge in macadamia nut production has been the inconsistent timing of abscission, which refers to the natural shedding of nuts from the tree (Trueman et al., 2002). Different cultivars exhibit varying abscission times, leading to the need for multiple harvesting cycles and increased labour (Gama et al., 2020; Trueman, 2003).

To address this challenge, researchers have explored the application of ethephon, a plant growth regulator with a known ability to induce uniform nut drop (Pratima and Chawla, 2019). When externally applied, ethephon triggers ethylene production, a plant hormone, and initiates ethylene-dependent processes, including the shedding of flowers or fruits (Torres et al., 2021). Although prior research, such as the work conducted by Aruwajoye et al., 2023 has predominantly concentrated on the preharvest effects of ethephon application on the biochemical components of macadamia nuts, there is limited research on the influence of ethephon on physical attributes of roasted nuts. Roasted macadamia nuts are known to possess

distinct quality characteristics compared to their raw nuts. Moreover, the quality of the kernel is mainly assessed based on its visual attributes, including size, shape, colour, texture, and shelf life (Pannico, 2014).

Despite the in-depth research on macadamia nut production and the application of ethephon, a research gap exists regarding its influence on the physical attributes of roasted macadamia nuts. Our study aims to bridge this knowledge gap, providing valuable insights into the impact of ethephon on the physical attributes of roasted macadamia nuts. As a result, the primary objective of this study is to evaluate the effect of preharvest ethephon application on the physicochemical and biochemical attributes of two macadamia cultivars, namely ‘788’ and ‘Beaumont’, during postharvest storage.

## **5.3 Materials and methods**

### ***5.3.1 Ethephon spray***

In Camperdown, KwaZulu-Natal, South Africa, macadamia nut varieties ‘Beaumont’ and ‘788’ were harvested from the Fyvie estates. To facilitate nut detachment from the trees, a plant growth regulator, ethephon 480 SL® was used. The ‘788’ trees were treated with 13.33 mL per liter per hectare, while the ‘Beaumont’ trees received 16.67 mL per liter per hectare, both administered using a Cima mist blower. This application of ethephon was focused on mature nuts, and nuts that naturally fell to the ground were considered successfully detached.

### ***5.3.2 Nut collection***

Macadamia nuts were collected from both treated and untreated trees of each cultivar, and they were classified into four groups based on their origin and handling: nuts that naturally fell from untreated trees (CD), nuts manually picked from untreated trees (CT), nuts

that naturally fell from trees treated with ethephon (ED), and nuts picked from ethephon-treated trees (ET). Following the harvest, the nuts were mechanically dehusked within 24 hours. After dehusking, the nuts were weighed and subjected to a controlled drying process. The drying process adhered to a specific temperature schedule, beginning at 35°C for 2 days, 45°C for 2 days, and 57°C for 2 days. This temperature regimen was adapted Gama et al., 2020, to enhance kernel recovery and overall quality.

### ***5.3.3 Roasting Process and Storage Conditions***

The experimental design for the study involved two main factors: cultivar and postharvest treatment. Under the cultivar factor, two varieties of macadamia nuts, ‘Beaumont’ and ‘788’, were considered. Each variety was further subdivided into ethephon-treated trees (ET) and control trees (CD) to assess the effects of the postharvest treatment. The postharvest treatment factor focused on the application of ethephon spray, with both treated and untreated trees being included in the study. Macadamia kernels from each treatment underwent a standardized roasting procedure, following the guidelines outlined by Aruwajoye et al. (2023). In summary, the nuts were roasted at 125°C for 15 minutes using a conventional hot air oven. Following roasting, the nuts were allowed to cool before being individually packed in polystyrene bags, with distinctive packaging for each treatment group (ED, ET, CT, and CD). The packed samples were then stored in a controlled storage chamber at a constant temperature of 25°C. Sampling intervals were established at 14-day intervals over a 56-day period, ensuring a thorough assessment of the attributes (Kernel weight, diameter, length,  $L^*$ ,  $a^*$ ,  $b^*$ ,  $h^\circ$ , and texture) of the roasted macadamia nuts throughout the storage duration. This structured approach ensured a comprehensive evaluation of the impact of cultivar and postharvest treatment on macadamia nut attributes.

### 5.3.4 Kernel weight, diameter, and length

In order to evaluate the physical characteristics of macadamia kernels across various treatments (CD, CT, ET, and ED), ten kernels per treatment were measured. The mass of the macadamia kernels was assessed in grams using a calibrated weighing scale (RADWAG Wagi Electronic Inc., Poland). Additionally, the diameter and length of each kernel were measured in millimeters (mm) employing a Mitutoyo Digimatic digital caliper (Mitutoyo Digimatic, Japan).

### 5.3.5 Colour measurement

Colour properties of 10 macadamia kernels were assessed using a Konica Minolta Chroma Meter CR-400 colorimeter. Three measurements were taken at different positions on each kernel, and the device was calibrated using a white standard tile. The parameters obtained included  $L^*$ ,  $a^*$ ,  $b^*$ , and  $h^\circ$ , with the  $h^\circ$  value presented in Equation 2. The  $L^*$  value signifies the degree of lightness of the macadamia kernels, with 0 indicating black and 100 indicating white. A positive  $a^*$  value reflects redness, while a negative value indicates a green hue, in line with previous studies (Wall & Gentry, 2007; Becerril-Sánchez et al., 2021). Positive  $b^*$  values correspond to a yellow colouration, while negative values suggest a bluish hue, as observed in studies by Becerril-Sánchez et al. (2021) and Thewes et al. (2022). Each treatment involved the evaluation of ten macadamia kernels.

$$h^\circ = \begin{cases} \operatorname{atan} \frac{b^*}{a^*} \frac{180^\circ}{\pi} & \text{if } a^* > 0 \text{ and } b^* \geq 0 \\ \operatorname{atan} \frac{b^*}{a^*} \frac{180^\circ}{\pi} + 180^\circ & \text{if } a^* < 0 \end{cases} \quad (2)$$

### **5.3.6 Texture analysis**

To assess the firmness of macadamia nuts subjected to different treatments (CD, CT, ET, and ED), we measured ten kernels per treatment. These measurements were conducted at 14-day intervals, starting from day 0 and continuing for 56 days. We employed a texture analyzer (TA.XT. Plus Texture Analyser, UK) with a shearing werner-Bratzler attachment. The procedure involved placing the kernel on a table beneath the descending blade, which cut through it at a consistent speed. Key test parameters included a pre-test speed of 2.0 mm/s, a test speed of 2.0 mm/s, and a down-stroke distance of 30.0 mm. The resistance of the kernel to shearing was recorded at 0.01-second intervals and presented in a force-deformation plot generated by a computer. The firmness value was identified as the maximum peak of the first compression (N) in the force-time curves (De Huidobro et al., 2005; Latha et al., 2012).

### **5.3.7 Data analysis**

Statistical analysis of variance (ANOVA) was executed using GenStat® 20th Edition (VSN International Ltd., Hertfordshire, UK), and the means were distinguished via the least significant difference (LSD) test at a 5% significance level. Python programming was employed to conduct a comprehensive evaluation of the influence of ethephon on the postharvest quality of macadamia nuts, involving principal component analysis (PCA), the generation of biplot diagrams, and the creation of a correlation matrix heatmap.

## **5.4 Results and Discussion**

### **5.4.1 Kernel weight, diameter, and length**

When assessing macadamia nuts, Falconer (1989) recommends an optimal kernel weight falling within the range of 2 to 3 g. This is based on the observations made by Hardner et al. (2001), who emphasized potential challenges in roasting for kernels below 1.5 g and

those exceeding 3.5 g. When examining the '788' cultivar, significant variability in nut mass among treatments is evident, with recorded mass values for CT, CD, ET, and ED at 3.62, 3.64, 2.28, and 2.25 g, respectively, and significantly higher values for CT and CD nuts ( $p < 0.05$ ). Conversely, the 'Beaumont' cultivar shows no significant differences in mass values for CT, CD, ET, and ED, measuring at 2.70, 2.73, 2.74, and 2.85 g, respectively. Notably, all treatments fall within the desirable kernel weight range, except for CT and CD in the '788' cultivar. While the significant differences in nut mass between '788' and 'Beaumont' may be attributed to cultivar-related distinctions as well as cultivar-specific response to ethephon, it's essential to note that ethephon application in our study was on mature nuts. This critical detail suggests that the observed variations within the same cultivar, influenced by ethephon, might be unique to the maturity stage at which the treatment was applied. Ries et al. (2020) support this, illustrating that ethephon treatment on mature nuts could yield nuanced impacts on fruit characteristics, further emphasizing the relevance of considering the maturity stage in evaluating ethephon's influence on macadamia nut mass. In the '788' cultivar, both control treatments (CD and CT) displayed significantly larger diameters compared to the nuts treated with ethephon (Table 5.1). However, no significant differences in diameter were noted among all treatments for the 'Beaumont' cultivar.

**Table 2.1** The effect of ethephon foliar application on the physiochemical parameters of roasted macadamia nuts.

	'788'				'Beaumont'			
	CT	CD	ET	ED	CT	CD	ET	ED
<b>Parameters</b>								
Mass	3.62±0.14 c	3.64±0.22 c	2.28±0.06 ab	2.25±0.08 a	2.70±0.10 ab	2.73±0.11 ab	2.74±0.12 ab	2.85±0.07 b
Diameter	19.82±0.29 cd	20.76±0.47 d	14.05±0.96 ab	17.05±0.38 b	17.95±0.26 bc	18.20±0.28 bc	18.40±0.52 bc	18.76±0.27 bcd
Length	11.89±0.25 a	14.03±0.51b	15.05±0.24bc	15.05±0.11bc	15.35±0.26 bc	15.45±0.29 c	15.05±0.29bc	15.05±0.22 bc

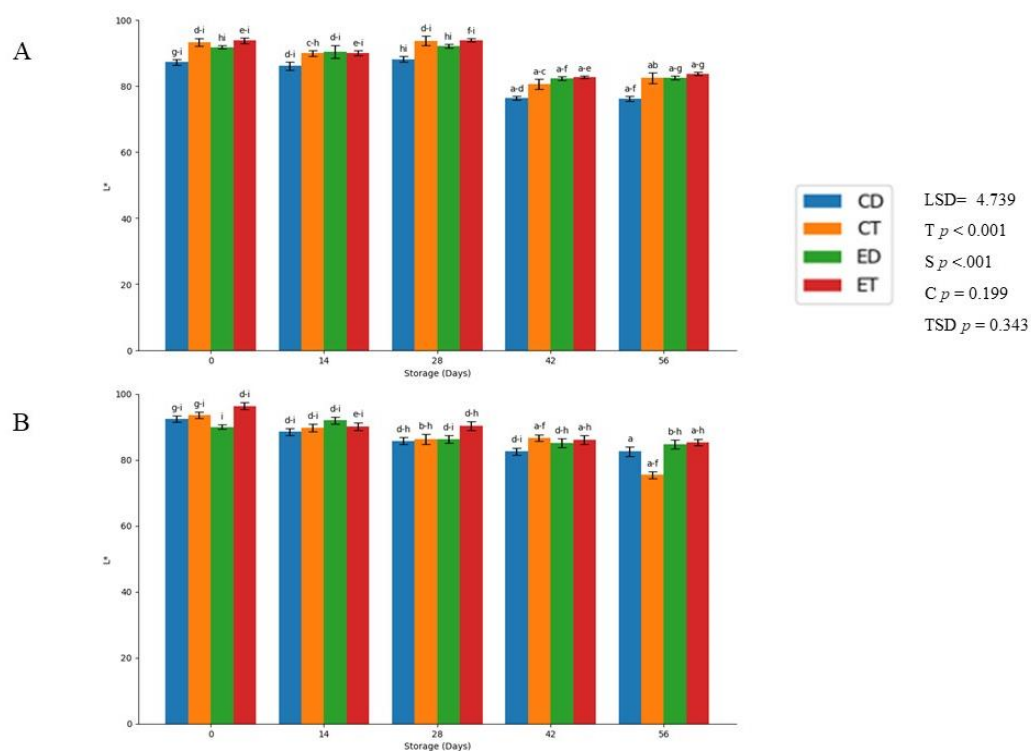
CD (control drop), CT (control tree), ET (ethephon tree), ED (ethephon drop). Values are the mean ± SE. Lowercase letters (a–d) indicate differences between treatments during storage.

### 5.4.2 Colour

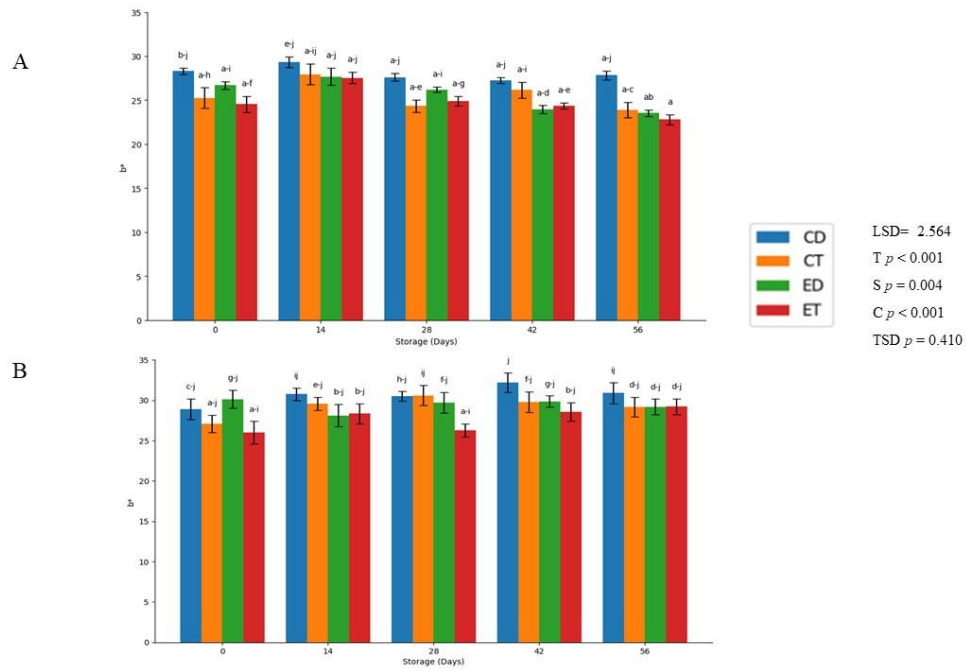
The colour of roasted macadamia kernels is a pivotal quality factor, contributing to a desirable light golden hue, a nutty flavour, and a crisp texture post-roasting (Ripperton et al., 1938). In Figures 5.1, 5.2, 5.3, and 5.4, the impact of treatment (harvest method), storage, and cultivar on L\*, a\*, b\* values, and hue angle of roasted macadamia nuts during accelerated storage at 25°C is presented. Both treatment and storage time significantly influenced the L\* value of the nuts ( $p < 0.001$ ), while the interaction between treatment, storage time, and cultivar showed no significant effect on the L\* value. For the '788' cultivar, the L\* value ranged from 86.00 to 91.70, and for the 'Beaumont' cultivar, it ranged from 75.40 to 96.35. Previous studies on roasted macadamia nuts reported L\* values ranging from 81.18 to 84.58 (Wall and Gentry, 2007; Phatanayindee et al., 2012). In the 'Beaumont' cultivar, all treatments exhibited a decrease in lightness (L\*) over time, with treatment CD and CT showing a significant decrease from day 0. Notably, the Control tree had the lowest L\* value at 75.40, indicating a darker colour compared to other treatments. However, all L\* values remained above the acceptable external L\* values for macadamia nuts (74.3) and the L\* value for kernels with external browning (55.3) (Wall and Gentry, 2007). The units for L\*, a\*, and b\* values, representing colour attributes, range from 0 to 100, with higher L\* values indicating lighter colours. It is important to note that these colour values play a critical role in determining the visual appeal of roasted macadamia nuts.

Over the storage period, the b\* values in both cultivars ranged from 22.78 to 32.18, signifying substantial variability in the response variable across different treatments. Roasting time also led to an increase in b\* values; for instance, Birch et al. (2010) found that roasting macadamia nuts for 30 minutes increased the b value from 22.76 to 30.02. In our study,

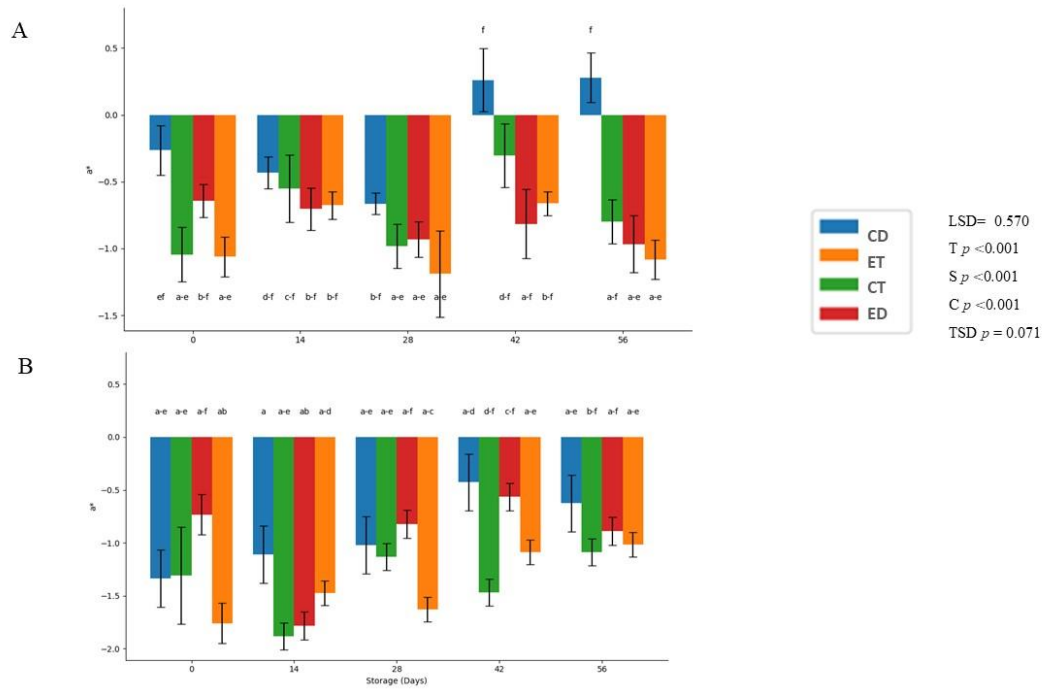
treatment, storage time, and cultivar all exerted a significant effect on the  $b^*$  value. In the ‘Beaumont’ cultivar at day 42, Control drop had the highest  $b^*$  value (32.18), corresponding with the low  $L^*$  values observed in the control drop. Regarding  $a^*$  values, significant differences in treatment, storage, and cultivar were observed. Specifically, in the ‘Beaumont’ cultivar, a gradual increase in the  $a^*$  value was noted over storage time. These results not only provide insights into the colour dynamics of roasted macadamia nuts but also hold implications for consumer preference and overall product quality.



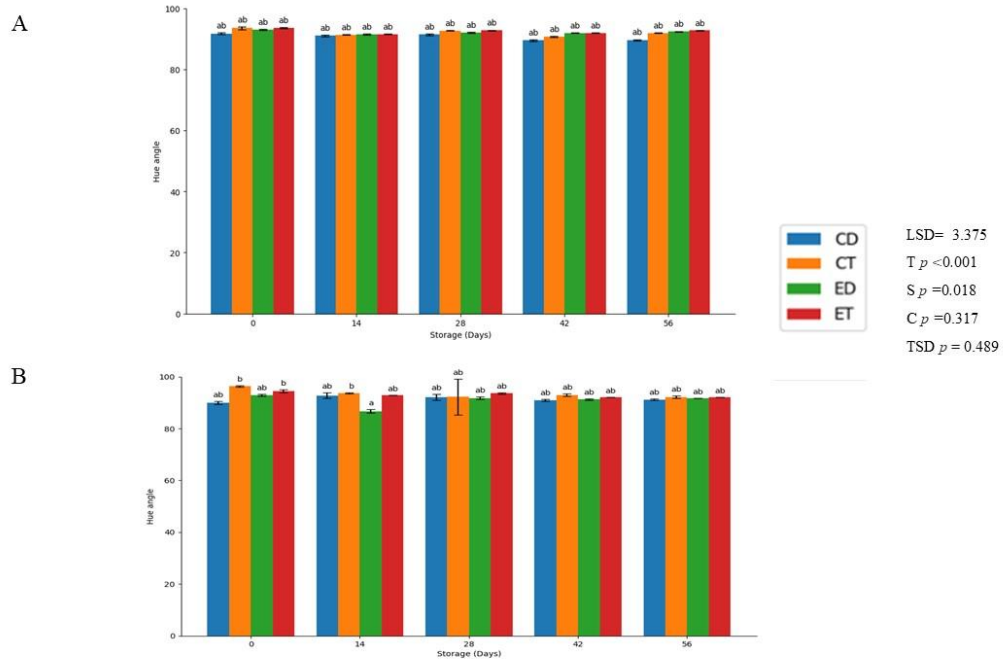
**Figure 5.1** The impact of ethephon application on the  $L^*$  values of A) ‘788’ and B) ‘Beaumont’ macadamia cultivars during storage is presented. The data is depicted as mean  $\pm$  standard errors derived from three replicates, with vertical bars indicating the standard errors of the means. T denotes treatment (ET, ED, CD and CT), C represents cultivar, and S stands for storage duration in days.



**Figure 5.2** The impact of ethephon application on the  $b^*$  values of A) ‘788’ and B) ‘Beaumont’ macadamia cultivars during storage are presented. The data is depicted as mean  $\pm$  standard errors derived from three replicates, with vertical bars indicating the standard errors of the means. T denotes treatment (ET, ED, CD, and CT), C represents cultivar, and S stands for storage duration in days.



**Figure 5.3** The impact of ethephon application on the  $a^*$  values of A) ‘788’ and B) ‘Beaumont’ macadamia cultivars during storage are presented. The data is depicted as mean  $\pm$  standard errors derived from three replicates, with vertical bars indicating the standard errors of the means. T denotes treatment (ethephon or control), C represents cultivar, and S stands for storage duration in days.

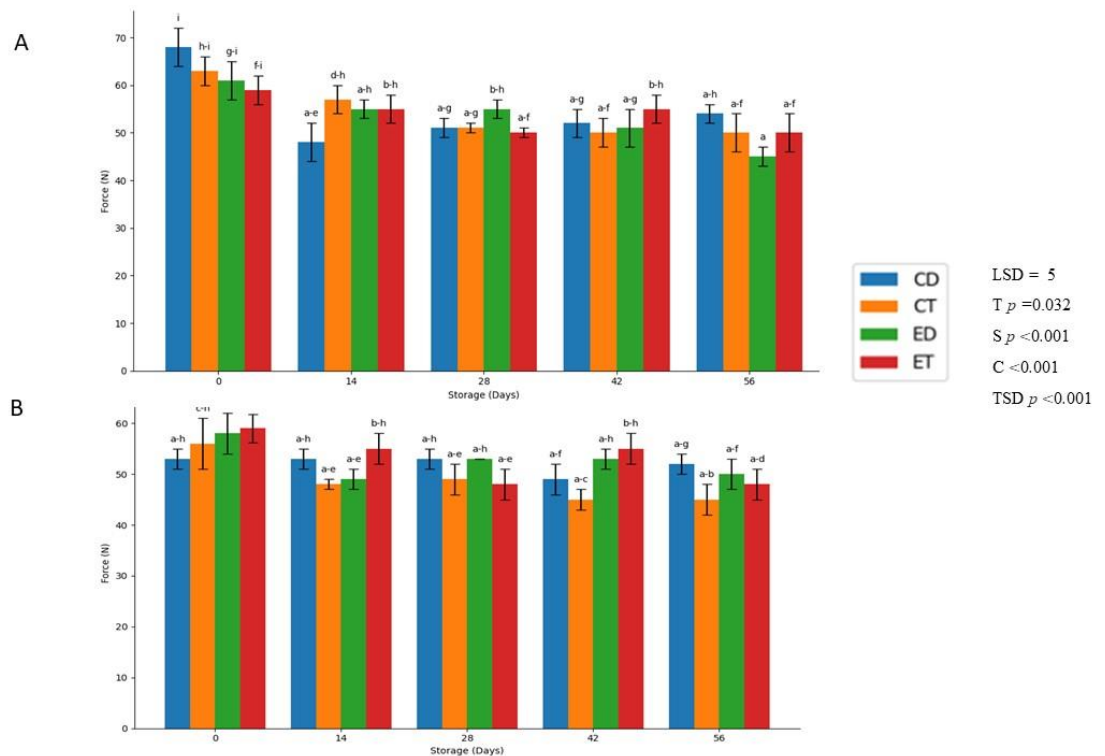


**Figure 5.4** The impact of ethephon application on the hue angle of A) ‘788’ and B) ‘Beaumont’ macadamia cultivars during storage are presented. The data is depicted as mean  $\pm$  standard errors derived from three replicates, with vertical bars indicating the standard errors of the means. T denotes treatment (ethephon or control), C represents cultivar, and S stands for storage duration in days.

### 5.4.3 Texture

Texture serves as one of the crucial determinants of nut quality (Ghirardello et al., 2013). The analysis of macadamia nut firmness revealed highly significant variations (fig 5.5) influenced by storage time, cultivar, and their combined interaction ( $p < 0.001$ ). However, the effect of treatment was not found to be highly significant ( $p = 0.03$ ) compared to cultivar and storage. Our results align with the findings of Moscetti et al. (2013), who reported that maintaining firmness is optimal at 4 °C, while decreases were observed at higher temperatures, such as 10 °C (Moscetti et al., 2013). A general decrease in firmness over storage was observed in both cultivars regardless of the treatments applied. Therefore, the

significant decreases in firmness can be attributed to the storage temperature (25°C) the nuts were subjected to.

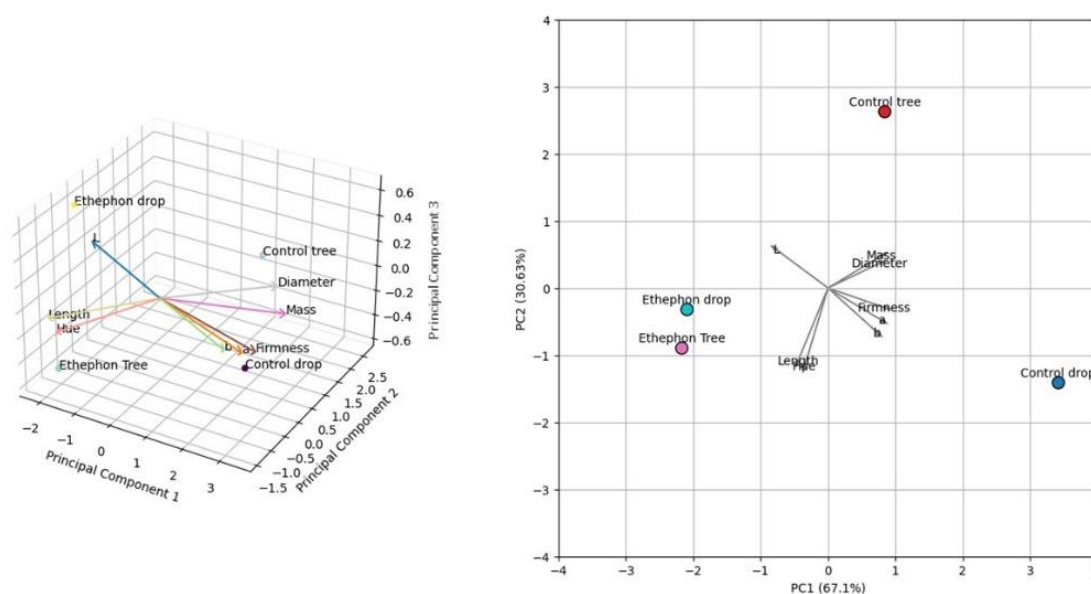


**Figure 5.5** The impact of ethephon application on the firmness (N) of A) ‘788’ and B) ‘Beaumont’ macadamia cultivars during storage are presented. The data is depicted as mean  $\pm$  standard errors derived from three replicates, with vertical bars indicating the standard errors of the means. T denotes treatment (ethephon or control), C represents cultivar, and S stands for storage duration in days.

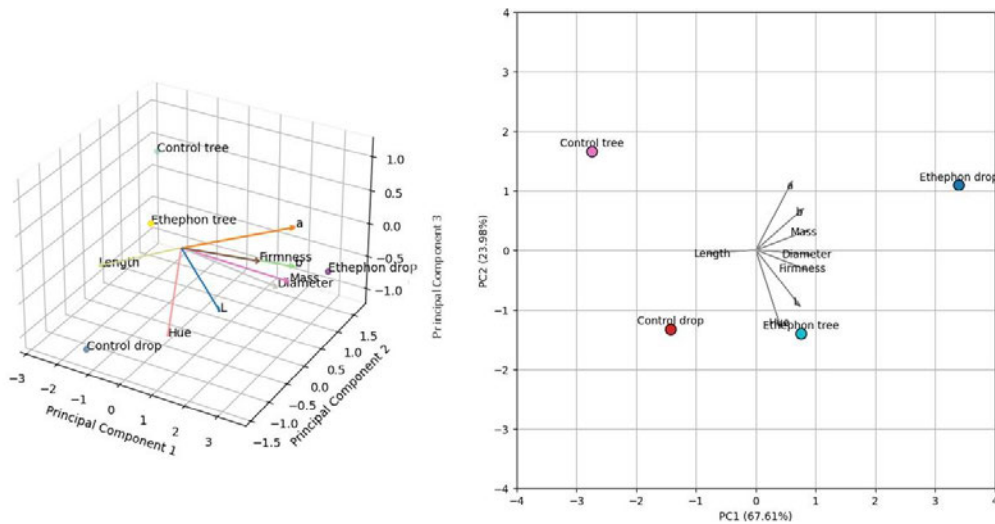
#### 5.4.4 PCA analysis and correlation matrix

Principal Component Analysis (PCA) biplots in both 2D and 3D were conducted to explore the treatment that predominantly influences the physical attributes of macadamia nuts. The present study analyzed the principal components of the evaluated physical attributes in the ‘Beaumont’ and ‘788’ cultivars using various visualization techniques, including a PCA biplot and PCA 3D plots (Figure 5.6 and 5.7), and a heat map (Figure 5.8). The considerable

percentages of total variation explained by PCA 1 and PCA 2 in both cultivars indicate the substantial variability in physical attributes. This suggests that specific treatments have a pronounced impact on the measured parameters. In the case of the ‘788’ cultivar, the PCA biplot analysis indicates PCA 1 explains 67.1% of the total variation, while PCA 2 accounts for 30.63% of the variability. It indicates that among all treatments CD had the highest firmness,  $a^*$  and  $b^*$  values. The mass and diameter were closely related to CT and the Length and Hue angle were related to ET. In the ‘Beaumont’ cultivar, PCA 1 accounted for 67.61% and PCA 2 accounted for 23.98% of the variability. ED displayed the highest  $a^*$ ,  $b^*$ , mass, and diameter of all the treatments while CD had the highest Hue angle.



**Figure 5.6** The biplot depicts the relationship between physical parameters and various treatments for the ‘788’ cultivar after postharvest processing. Subfigures depict the projection of Principal Component 1 (PC1) versus Principal Component 2 (PC2) in 3D and 2D, respectively.



**Figure 5.7** The biplot depicts the relationship between physical parameters and various treatments for the ‘Beaumont’ cultivar after postharvest processing. Subfigures depict the projection of Principal Component 1 (PC1) versus Principal Component 2 (PC2) in 3D and 2D, respectively.

The correlation matrix served as a valuable tool to assess the linear relationships among parameters, with values ranging from -1 to +1. Notably, mass exhibited a robust correlation with both diameter and firmness across both cultivars. Specifically, for the ‘Beaumont’ cultivar, mass demonstrated a correlation of  $r=0.90$  with diameter and  $r=0.82$ , while in the ‘788’ cultivar, the correlations were even higher, with  $r=0.95$  with diameter and  $r=0.72$  with firmness. Moreover, both cultivars exhibited strong correlations in the  $a^*$  and  $b^*$  values, with  $r=0.98$  in ‘Beaumont’ and  $r=0.91$  in the ‘788’ cultivar. This indicates the significant associations among these physical attributes in the studied macadamia cultivars



**Figure 5.8** The correlation matrix of the physical attributes of macadamia for both: A) ‘Beaumont’ and B) ‘788’ cultivar

## 5.5 Conclusions

This study sheds light on the intricate interplay of factors influencing the physical characteristics of macadamia nuts, focusing specifically on the '788' and 'Beaumont' cultivars. Significant variations in nut mass, diameter, colour attributes, and texture emerged across diverse treatments and storage conditions. Particularly in the 'Beaumont' cultivar, ED displayed a significant impact on influencing attributes such as  $a^*$ ,  $b^*$ , mass, and diameter. For the '788' cultivar, Principal Component Analysis (PCA) highlighted that CD treatment exhibited the highest values for firmness,  $a^*$ , and  $b^*$ . These findings show the importance of recognizing cultivar-specific responses, suggesting that the efficacy of treatments can vary based on the unique characteristics of each cultivar. The nuanced effects observed in this research emphasize the need for a tailored approach in post-harvest interventions for macadamia nuts. Understanding the differential responses of cultivars to ethephon is crucial for optimizing production practices and ensuring the desired quality attributes. Overall, this study contributes valuable insights into the complex dynamics influencing macadamia nut quality, providing a foundation for informed decision-making in the macadamia industry.

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**CHAPTER 6: IMPROVING THE QUALITY OF MACADAMIA NUTS  
HARVESTED WITH ETHEPHON: MODELING AND OPTIMIZING DRYING  
AND STORAGE CONDITIONS**

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## 6.1 Abstract

The macadamia nut is a nutritional crop experiencing continuous global growth in production. Despite the numerous health benefits associated with macadamia nuts, their economic advantage is significantly hampered by inadequate storage and drying conditions, resulting in substantial postharvest losses. This study focuses on modelling the firmness and colour parameters of macadamia nuts from the '788' and 'Beaumont' cultivars against drying days and storage conditions. The aim is to predict the minimal processing requirements for achieving satisfactory postharvest nut appearance. The investigated input parameters include storage temperature, drying temperature, and storage days, while firmness and colour parameters ( $L^*$ ,  $a^*$ ,  $b^*$ , and  $h^\circ$ ) serve as the resultant outputs. The range of the input parameters spans from  $-22^\circ\text{C}$  to  $25^\circ\text{C}$  for storage temperature,  $35$  to  $57^\circ\text{C}$  for drying temperature, and  $0$  to  $70$  days for storage duration. The Box Behnken design of experiments was employed to generate 17 experimental runs for each output parameter of the models. The resulting models demonstrated significance, with high coefficients of determination ranging between  $0.83$  and  $0.97$ . Notably, maximum  $L^*$  values of  $76.25$  and  $79.75$  for the '788' and 'Beaumont' cultivars, respectively, were achieved under reduced drying days and optimum storage temperature. The firmness model proved to be significant ( $P < 0.0001$ ) specifically for the '788' cultivar. In conclusion, this study provides a theoretical framework, presenting optimal set points crucial for the effective preservation of the '788' and 'Beaumont' varieties of macadamia nuts against external discolouration. This framework aims to enhance the quality and overall market value of these macadamia nut varieties.

**Keywords:** Macadamia nuts, drying, storage, modelling, colour, firmness

## 6.1 Introduction

Macadamia nuts are known due to their nutritional profile, characterized by a high content of monounsaturated fatty acids (constituting 59% of total fat) and dietary fiber (Buthelezi et al., 2019; Hu et al., 2019). Adopting a healthy lifestyle, one that incorporates a diet abundant in unsaturated fats and dietary fiber offers various health benefits (Durko et al., 2014; Ruiz et al., 2014). However, their high monounsaturated fatty acid content makes them prone to oxidative reactions and subsequent rancidity (Shahidi & John, 2013). Hydrolytic rancidity increases with higher moisture content, creating an environment conducive to microbial and lipolytic enzyme activity (Buthelezi et al., 2021). Therefore, drying is a critical process to alleviate rancidity challenges affecting macadamia nut quality (Minh et al., 2018). Macadamia nuts undergo drying until they reach a moisture content of 3.5% within the shell, aiming to achieve a kernel moisture content of 1.5% (Poogungploy et al., 2018). Various methods exist, with the most commonly cited involving drying dehusked nuts at temperatures of 37 °C for 2 days, 45 °C for 2 days, and 57/58 °C for 2 days (Meyers et al., 1999; Richards et al., 2020; De Silva et al., 2022; Gama et al., 2020). Improper drying can result in defects such as kernel browning, which intensify over time and with temperature variations (Walton et al., 2013). Hence, storage temperature and duration significantly impact nut quality, as higher temperatures accelerate biological reactions leading to a decline in nut integrity (Mashau et al., 2012; Gama et al., 2020). Ensuring product quality is crucial, especially regarding external attributes like colour, shape, and size, which play a vital role in convincing consumers to make an initial purchase (Magwaza and Opara, 2015). Therefore, determining optimal storage conditions is essential to meet the rising demand for high-quality macadamia nuts.

Process modeling and optimization studies that evaluate drying temperature, storage temperature, and period parameters can be advantageous. Response Surface Methodology (RSM) modeling is a beneficial tool for understanding both individual and interactive effects of crucial parameters on macadamia nuts during postharvest storage (Aruwajoye et al., 2017). This approach aids in developing mathematical models between fruit quality parameters and multiple storage conditions, facilitating the optimization of process parameters to moderate undesirable defects. For instance, a study by Liu et al. (2021) used RSM to optimize drying conditions for purple cabbage, addressing postharvest decay challenges. The RSM has also been employed to predict the evolution of tomato colour concerning storage temperature and humidity (Han et al., 2022). Notably, the traditional one factor at a time method provides limited information and cannot predict how specific quality parameters perform individually and in interaction with others in terms of postharvest quality output (Aruwajoye et al., 2017; Han et al., 2022).

In this study, we investigated the singular and interactive impacts of storage temperature, drying days, and storage period on the colour and firmness of macadamia nuts harvested with ethephon ('788' and 'Beaumont' cultivars) through the application of Response Surface Methodology (RSM). Additionally, we aimed to optimize output parameters, including  $L^*$ ,  $a^*$ ,  $b^*$ , Hue angle, and firmness, which collectively represent colour and texture for both cultivars. The goal was to attain the most favorable values by considering all input parameters in the optimization process.

## 6.2 Materials and methods

### 6.2.1 Nut collection and preparation

The macadamia nut cultivars ‘Beaumont’ and ‘788’ were harvested from Fyvie Estates, uMlaas Road, Camperdown, KwaZulu-Natal, South Africa (latitude: 29°47'50.3" S, longitude: 30°27'54" E). The harvest took place in May 2022. To facilitate nut abscission in macadamia trees, Ethephon 480 SL®, applied as ethylene, was utilized on mature macadamia nuts. The concentration of ethephon adhered to industry standards, with ‘788’ trees receiving 13.33 mL/L and 'Beaumont' trees receiving 16.67 mL/L. Both doses were applied using a Cima mist blower for effective nut abscission. Nuts that were successfully abscised were collected and utilized for further analysis.

### 6.2.2 Drying and cracking processes

Freshly harvested macadamia nuts were dehusked mechanically within 24 h. The maximum drying temperature was selected according to industry drying temperature. For the purpose of this study, the minimum, average, and maximum of the selected drying temperature was used as indicated in Table 6.1. Nuts were cracked after each selected temperature and identified according to the total number of drying days (2, 4, and 6).

**Table 3.1** Macadamia nuts drying temperature ranges and duration.

Drying capacity	Drying temperature and days	Total number of drying days
Minimum	35 °C for 2d	2
Average	35 °C 2d and 45 °C 2d	4
Maximum	35 °C 2d ,45 °C 2d and 57 °C 2d	6

### 6.2.3 Experimental design and storage conditions

Optimization of the colour of macadamia nuts was investigated using the Response surface methodology (RSM). The input parameters investigated include drying days, storage temperature, and storage period while the output parameters were  $L^*$ ,  $a^*$ ,  $b^*$ , and  $h^\circ$ . The range of the parameters was  $-22$  to  $25^\circ\text{C}$  (storage temperature), 2 to 6 drying days, and 7 to 70 (storage period) respectively (Table 6.2). Box Behnken design of experiments was used to generate 17 experimental runs for the model. Each experimental run had 5 kennels of macadamia. The experimental data was fitted in the quadratic polynomial model equation, relating the input variables to the output response. The general form of the model is shown in Eq 1.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

$Y$  represents the output response (i.e.  $L^*$ ,  $a^*$ ,  $b^*$ , and  $h^\circ$ ),  $\beta_0$  is the intercept,  $\beta_1 X_1$  to  $\beta_3 X_3$  represents the linear blending portion,  $\beta_{11} X_1^2$  to  $\beta_{33} X_3^2$  are the interaction coefficients. The fitness of the model was assessed using the Analysis of Variance (ANOVA) using Design-Expert Version 8 (Stat-Ease, Inc., USA).

**Table 6.2** Experimental design

Factors	Factor levels		
	-1	0	-1
Drying days	2	4	6
Storage temperature ( $^\circ\text{C}$ )	-22	1.5	25
Storage period (days)	7	38.5	70

#### ***6.2.4 Development of polynomial equations***

In summary, polynomial equations were developed for the colour parameters ( $L^*$ ,  $a^*$ ,  $b^*$ , and  $h^\circ$ ) and firmness of the macadamia nuts as follows: experimental data on macadamia nut storage and processing conditions, including storage temperature, storage period, and drying days, as outlined in Tables 6.3 and 6.4 for the ‘Beaumont’ and ‘788’ cultivars, were obtained. Subsequently, the data were analyzed to establish relationships between the output parameters (colour and firmness of the nuts) and their corresponding independent variables (storage temperature, storage period, and drying days). The data were then used to fit the polynomial equations, as expressed in Equation 1. It was confirmed that the polynomial equation was able to capture the observed trends in the data, as shown by the results of the model evaluation ( $R^2$ ).

#### ***6.2.5 Determination of colour***

The external colour of fresh and dried raw macadamia kernels was measured on all samples subjected to different treatment on each data recording interval (Table 6.3). Measurements were done using a colourimeter (Konica Minolta Chroma Meter CR-400) by averaging three measurements taken around three kernel positions. Calibration of the device was done with a white standard tile. The determined parameters were  $L^*$ ,  $a^*$ , and  $b^*$  and  $h^\circ$ . The  $h^\circ$  is presented in Eq.2. The  $L^*$  value indicates the degree of lightness of macadamia kernels (0 = black, 100 = white), while positive  $a^*$  indicates the degree of redness and negative  $a^*$  indicates the intensity of green (Wall & Gentry, 2007; Becerril-Sánchez et al., 2021). Positive  $b^*$  values indicate yellow and negative indicates blue (Becerril-Sánchez et al., 2021; Thewes et al., 2022). Five macadamia kernels were used for each experimental run.

$$h^{\circ} = \begin{cases} \text{atan} \frac{b^*}{a^*} \frac{180^{\circ}}{\pi} & \text{if } a^* > 0 \text{ and } b^* \geq 0 \\ \text{atan} \frac{b^*}{a^*} \frac{180^{\circ}}{\pi} + 180^{\circ} & \text{if } a^* < 0 \end{cases} \quad (2)$$

### 6.2.6 Texture analysis

The firmness of macadamia nuts was measured using a texture analyzer (TA.XT. Plus Texture Analyser, UK). A shearing werner-Bratzler was attached. The kernel was placed on the table under the blade and was cut through as the blade moved down with constant speed. The assay parameters were pre-test speed: 2.0 mm/s and test speed: 2.0 mm/s. Downstroke distance was: 30.0 mm. The resistance of the kernel sample to shearing was recorded every 0.01 s and plotted by a computer in a force–deformation plot. The maximum peak of the first compression (N) in the force-time curves indicates the firmness value (De Huidobro et al., 2005; Latha et al., 2012). Firmness was measured for all the experimental runs for both cultivars.

## Results and discussion

### 6.4.1 Development of RSM models

Macadamia nut storage and processing conditions with the corresponding colour output parameters are presented in Tables 6.3 and 6.4 for the two different cultivars. The experimental data were used to develop up to four polynomial equations for each macadamia nut cultivar ('Beaumont' and '788') relating to colour ( $L^*$ ,  $a^*$ , and  $b^*$  and  $h^{\circ}$ ) shown in Equations (3), (4), (5), (6) respectively for 'Beaumont' and (7), (8), (9) and (10) respectively for '788'. Firmness is shown in equation (11).

$$L^* = 82.22 + 6.79A + 0.035B + 0.87C + 2.02AB - 1.3AC - 0.15BC - 4.20A^2 + 1.26B^2 - 2.91C^2 \quad (3)$$

$$a^* = -1.19 - 0.5A + 0.06B + 0.19C + 0.087AB - 0.12AC + 0.11BC + 0.32A^2 + 0.25B^2 - 0.17C^2 \quad (4)$$

$$b^* = 22.04 - 0.54A - 0.91B - 0.19C - 0.42AB - 0.25AC + 0.80BC - 2.06A^2 - 0.30B^2 + 0.95C^2 \quad (5)$$

$$h^\circ = 93.20 + 0.7A + 0.25B - 0.25C + 0.7AB + 0.25AC - 0.25BC - 0.48A^2 - 0.98B^2 + 0.025C^2 \quad (6)$$

$$L^* = 71.32 + 8.09A - 3.26B - 1.71C + 2.63AB + 1.66AC - 2.44BC - 2.97A^2 + 1.91B^2 + 1.73C^2 \quad (7)$$

$$a^* = -0.86 + 0.56A + 0.086B + 0.11C - 0.039AB + 0.15AC + 0.26BC - 0.46A^2 - 0.063B^2 - 0.24C^2 \quad (8)$$

$$b^* = 22.74 + 3.44A - 1.92B - 0.59C + 1.40AB + 1.40AB + 1.69AC + 0.52BC - 2.34A^2 - 0.47B^2 + 3.06C^2 \quad (9)$$

$$h^\circ = 92.19 - 2.3A + 0.18B - 0.11C - 0.74AB - 0.93AC - 1.05BC + 2.26A^2 + 0.26B^2 - 0.078C^2 \quad (10)$$

$$\text{Firmness(N)} = 61.80 + 18.63A + 0B + 0.63C + 3.25AB + 1.50AC + 0.75 - 19.65A^2 - 0.40B^2 + 4.85C^2 \quad (11)$$

Where Y represents the output i.e. colour and firmness. A, B, and C represent drying days, storage temperature, and storage days respectively.

#### **6.4.2 Significance of the models**

For the 'Beaumont' cultivar, the Analysis of Variance (ANOVA) of the quadratic regression model for each of the colour parameters  $L^*$ ,  $a^*$ ,  $b^*$  and  $h^\circ$  gave F-values of 24.78,

6.89, 3.78, and 3.73 respectively and P-values of 0.0002, 0.0093, 0.0468, and 0.0482 respectively demonstrating the significance of the models ( $P < 0.05$ ). Similarly, analysis of the '788' model for each of the colour parameters  $L^*$ ,  $a^*$ ,  $b^*$  and hue angle ( $h^\circ$ ) gave F-values of 4.85, 4.26, 3.68, 4.95 respectively and P-values of 0.02, 0.04, 0.05 and 0.02 respectively demonstrating the significance of the models ( $P < 0.05$ ). Moreover, the quadratic regression model for firmness and sucrose gave F-values of 42.63 and 4.07 respectively, and P-values of 0.001 and 0.0388 respectively. The  $L^*$ ,  $a^*$ , and  $b^*$  and  $h^\circ$  models' coefficient of correlation ( $R^2$ ) for 'Beaumont' were 0.97, 0.90, 0.83, and 0.83 respectively, and 0.88, 0.85, 0.83 and 0.86 for the '788' respectively. The coefficient of correlation of firmness and sucrose were 0.98 and 0.84 respectively. Thus, the models can account for 83-97% and 72-98% of the variability observed in the outputs the 'Beaumont' and '788' respectively.

RSM predicted the evolution of macadamia nuts' external colour, which has economic value and practical relevance in regulating the storage temperature of macadamia nuts that are dried at different temperatures. The results also showed that the quadratic term of drying days ( $A^2$ ) has a significant effect on 'Beaumont' colour parameters ( $L^*$ ,  $a^*$ , and  $b^*$ ). Drying time affects the colour appearance of macadamia nuts (Borompichaichartkul et al., 2009). Proper drying of the nuts reduces the moisture content that is required for microbial growth (Brar & Danylukr, microbial growth, 2018). Moreover, microbial growth and enzyme activity is facilitated at higher temperatures. Tree nuts are generally stored at lower temperatures to reduce the effect of metabolic reactions that facilitate the quality reduction in nuts and further become pronounced over time (storage period) (Gama et al., 2018; Bonazzi& Dumoulin 2011).

**Table 6.3** Box-Behnken design of the storage and drying conditions of ‘Beaumont’ and the colour parameter outputs.

Run	Drying days	Storage Temperature	Storage Period (Days)	L*		b*		a*		h°	
				Actual	predicted	Actual	predicted	Actual	predicted	Actual	predicted
1	4	-22	7.00	79.984	79.636	25.14	20.711	-1.184	-1.251	92	92.125
2	6	-22	38.50	84.034	84.761	19.952	20.469	-1.408	-1.275	91	91.375
3	2	1.5	7.00	64.580	65.983	21	21.411	-1,01	-0.846	93	93.250
4	6	25	38.50	87.068	88.123	17.942	17.803	-1,062	-0.965	93	93.375
5	2	-22	38.50	75.534	74.479	20.572	20.711	0	-0.097	92	91.625
6	2	25	38.50	70.486	70.758	20.246	19.729	0	-0.133	91	90.625
7	6	1.5	70.00	82.948	81.545	20.364	19.953	-1.302	-1.466	94	93.750
8	2	1.5	70.00	71.030	70.410	21.566	21.533	-0,284	-0.218	91	91.500
9	4	25	7.00	81.432	79.757	21.066	21.172	-1,302	-1.333	93	93.123
10	4	1.5	38.50	80.896	82.223	23.99	22.042	-0,896	-1.185	93	93.20
11	4	1.5	38.50	82.388	82.223	21.7	21.700	-1,394	-1.185	94	93.200
12	4	1.5	38.50	82.454	82.22	22.096	22.042	-1,466	-1.185	93	93.20
13	4	-22	70.00	80	81.675	22.728	22.622	-1,115	-1.084	92	91.875
14	6	1.5	7.00	81.874	82.494	20.79	20.823	-1,542	-1.608	94	93.500
15	4	1.5	38.50	83.500	82.223	21.55	22.042	-0,99	-1.185	93	93.00
16	4	25	70.00	80.848	81.196	21.84	22.390	-0,796	-0.729	92	92.00
17	4	1.5	38.50	81.876	82.223	20.876	22.042	-1,18	-1.185	93	93.200

**Table 6.4** Box-Behnken design of the storage and drying conditions of ‘788’ and the colour parameter outputs.

Run	Drying days	Storage Temperature	Storage Period (Days)	L*		b*		a*		h°	
				Actual	predicted	Actual	predicted	Actual	predicted	Actual	predicted
1	4	-22	7.00	78.66	77.071	26.464	25.363	-1,25	-1.547	92,708	93.406
2	6	-22	38.50	81.228	83.082	25.422	28.219	-0,942	-0.812	92,282	90.944
3	2	1.5	7.00	66.69	65.6344	23.082	20	-2,246	-1.954	95,688	95.944
4	6	25	38.50	77.242	75.814	21.762	24.379	-1,1	-0.508	93,03	91.303
5	2	-22	38.50	68.526	66.898	20.898	21.332	-1,73	-1.930	94,91	95.661
6	2	25	38.50	54	59.630	11.627	17.493	-1,73	-1.626	98,615	96.020
7	6	1.5	70.00	76.773	77.368	27.21	25.711	-0,555	-0.484	91,193	91.020
8	2	1.5	70.00	59.054	61.184	17.394	18.824	-2,384	-1.661	98,386	95.737
9	4	25	7.00	76.75	69.802	24.22	21.524	-1,344	-1.243	93,306	93.765
10	4	1.5	38.50	60.452	71.356	18.484	22.856	-1,816	-1.219	95,93	93.482
11	4	1.5	38.50	72.098	71.356	24.59	22.856	-0,68	-1.219	91,746	93.482
12	4	1.5	38.50	73.193	71.356	24.345	22.856	-0,8575	-1.219	92,035	93.482
13	4	-22	70.00	78.04	72.910	25.398	24.187	-1,49	-1.195	93,548	93.199
14	6	1.5	7.00	77.78	81.528	26.154	26.887	-1,022	-0.836	92,206	91.227
15	4	1.5	38.50	74.946	71.356	21.988	22.856	-0,548	-1.219	91,658	93.482
16	4	25	70.00	69.37	65.641	25.216	20.348	-0,022	-0.89	89,934	93.558
17	4	1.5	38.50	68.232	71.356	24.29	22.856	-1,005	-1.219	92,04	93.482

**Table 6.5** Box-Behnken design of the storage and drying conditions of ‘788’ firmness.

Run	Drying days	Storage Temperature	Storage Period (Days)	Firmness(N)	
				Actual	predicted
1	4	-22	7.00	70.00	70.15
2	6	-22	38.50	55.00	60.90
3	2	1.5	7.00	27.00	29.25
4	6	25	38.50	65.00	67.40
5	2	-22	38.50	25.00	22.60
6	2	25	38.50	22.00	16.10
7	6	1.5	70.00	70.00	67.75
8	2	1.5	70.00	29.00	35.05
9	4	25	7.00	65.00	68.65
10	4	1.5	38.50	62.00	61.80
11	4	1.5	38.50	63.00	61.80
12	4	1.5	38.50	64.00	61.80
13	4	-22	70.00	66.00	62.35
14	6	1.5	7.00	92.21	86.15
15	4	1.5	38.50	63.00	61.80
16	4	25	70.00	64.00	63.85
17	4	1.5	38.50	57.00	61.80

## 6.4.2. Interactive effects of process parameters

### 6.4.2.1 Colour

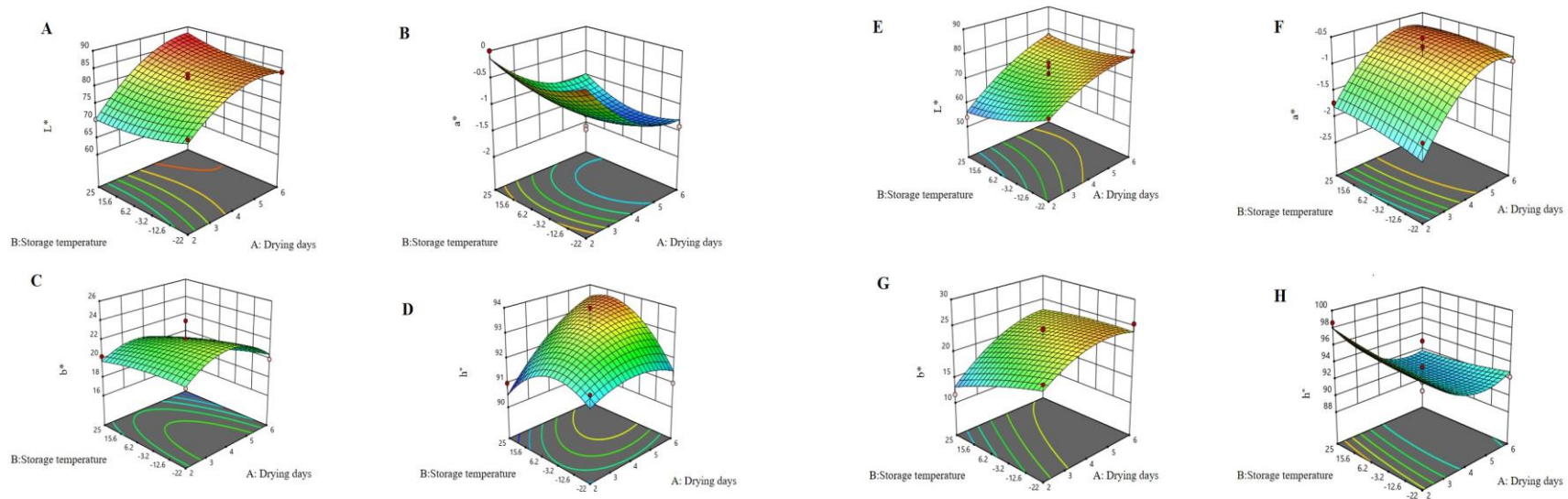
Colour parameters  $L^*$ ,  $a^*$ , and  $b^*$  and  $h^\circ$  of 'Beaumont' and '788' from various experimental runs are shown in Tables 2 and 3. 'Beaumont' colour parameters  $L^*$  ranged from 64.58 to 87.07, while  $b^*$  ranged from 17.94 to 22.62. Also,  $a^*$  ranged from -1.54 to 0, while  $h^\circ$  ranged from 90.63 to 93.75. On the other hand, '788'  $L^*$  ranged from 54 to 81.23, while  $b^*$  ranged from 11.63 to 27.21. Also,  $a^*$  ranged from -2.25 to -0.02, while the hue angle ranged from 90.44 to 96.02. Generally, Macadamia nuts should have a light cream colour and an acceptable external  $L^*$  value. In the macadamia industry, the acceptable external  $L^*$  value is 74.3, and an external  $L^*$  value of 55.3 is considered external browning (de Carvalho et al., 2018; Tan et al., 2020). Therefore, the  $L^*$  value is a good parameter for monitoring the colour change in macadamia nuts.

A view of the 3D response surface graphs of the interactive effects between the independent variables and response variables of 'Beaumont' and '788' are shown in Fig. 6.1 (A-H). Macadamia nuts were better preserved when stored at a lower storage temperature. From the display of the 3D graphs, increasing storage temperature from  $-22^\circ\text{C}$  to  $-10.5^\circ\text{C}$  while simultaneously increasing drying days from 2 to a maximum of 5 led to an  $L^*$  value increase from 56 to 70 Fig. 6.1 (A). Maintaining storage temperature at  $1.5^\circ\text{C}$  while increasing drying days from 3 to a maximum point of 5 led to a simultaneous increase in  $L^*$  value from 70 to 80. Improved drying was the main reason for the improved  $L^*$  value. A further increase in drying days beyond 5 days led to a decrease in  $L^*$  Fig. 6.1 (E). In a study where macadamia nuts were dried at  $30^\circ\text{C}$  for 7 days,  $40^\circ\text{C}$  for 7 days, and  $60^\circ\text{C}$  for 3 days, the  $L^*$  value decreased significantly (Phatanayindee et al., 2012). Therefore, prolonged drying could result in a reduction in lightness.

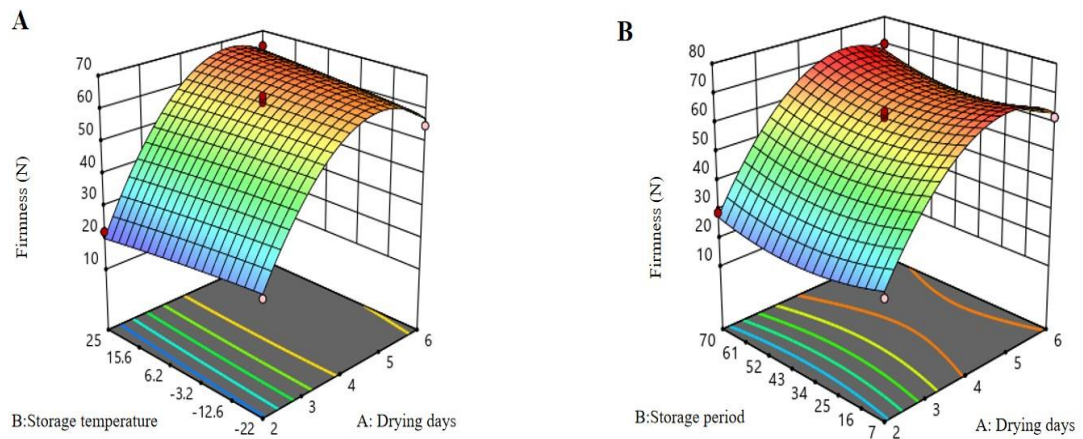
The response surface graphs also revealed that increasing storage temperature from -22°C to 1.5°C while simultaneously increasing drying days from 2 to 4 led to a simultaneous increase in  $h^{\circ}$  from 90.5 to 93.5 Fig. 6.1(D). Decreasing storage temperature from 25°C to -22°C while increasing drying days from 2 to 4 led to a decrease in  $h^{\circ}$  from 94.5 to 91.5. In cultivar '788', runs 1, 2, 4, 7, 13, and 14 (table 6.4) had  $L^*$  values that were higher than 74.3, and run 6 had an  $L^*$  value that was less than 55.3 which indicated external browning. Whilst for 'Beaumont' (table 6.3) all runs had an external  $L^*$  that is greater than 74.3 except for runs 2, 6, and 8 which were only dried for two days. Run 6 for both cultivars resulted in microbial growth. '788' displayed more severe symptoms. Microbial activity is expected at high moisture content and high storage temperatures because microbial growth is linked with water activity (Boaghi et al., 2019). However, microbial activity was not observed in nuts that were only dried for two days and stored at low temperatures. Tree nuts are normally stored at a lower temperature ranging from 4°C to 15°C to preserve quality (Gama et al., 2018).

#### **6.4.2.2 Firmness**

The kernel quality of macadamia nuts also includes a firm and crispy texture (Kader, 2013). In this study, the firmness model was only significant ( $P < 0.0001$ ) for cultivar '788' and the RSM graph is displayed in Fig. 6.2 (A-B). Results ranged from 22 N to 70 N (table 6.5). Macadamia nuts became more firmer as drying days increased Fig. 6.2 (A-B). Moreover, a maximum firmness was observed on day 5. Moisture content influences the firmness of nuts i.e., nuts that have a lower moisture content are associated with higher firmness (Wu et al., 2019). Hence the trend observed in Fig. 6.2 (A-B). However, after day 5 of drying, a decrease in firmness was noted indicating a reduction in kernel strength as similarly noted by Shakerardekani et al (2011) that the hardness of pistachio kernels reduced during roasting when temperature and time were increased (Shakerardekani et al., 2011).



**Figure 6.1** Response surface graphs showing the interactive effects of process and storage conditions on colour parameters



**Figure 6.2** Response surface graphs illustrating interactive effects of processing and storage conditions on firmness.

**Table 6.6** Validation of the optimized firmness and colour parameters models.

	'Beaumont'		'788'	
	Predicted	Actual	Predicted	Actual
<b>L*</b>	79.42	79.75±0.97	69.32	76.25±2.43
<b>a*</b>	-1.30	-1.60±0.18	-1.27	-1.33±0.31
<b>b*</b>	21.17	22.17±0.93	21.33	24.96±2.34
<b>h°</b>	93.05	94.13±0.57	93.89	93.02±0.47
<b>Firmness (N)</b>	-	-	64.63	50.52±1.60

Mean ± standard deviation.

### ***6.4.2.3 Validation of developed RSM models***

Validation experiments were conducted under storage elevated temperatures and reduced drying days to determine the optimal acceptable colour parameters (Table 6.5). The recommended conditions for drying the nuts were identified as 4 days, with subsequent storage at 25°C for approximately 7.95 days. The optimized conditions yielded outcomes consistent with the model-predicted results, except for the L\* values of '788', which were marginally higher (predicted = 69,32, actual = 76,25). While these L\* values exceeded the recommended thresholds for macadamia nuts (de Carvalho et al., 2018; Tan et al., 2020), our findings indicate the efficacy of process modeling in optimizing macadamia nut parameters. The results derived from the optimized Response Surface Methodology (RSM) can contribute to the dynamic grading of macadamia nuts, thereby enhancing their quality and overall market value. Additionally, this approach may serve as a strategy to reduce energy consumption during drying processes by minimizing drying duration.

### ***6.5 Conclusion and Recommendations***

The present study aimed to optimize postharvest parameters, specifically firmness and colour attributes (L\*, a\*, b\*, and h°), for the '788' and 'Beaumont' varieties of macadamia nuts to achieve enhanced quality. The developed response surface methodology models exhibited high coefficient of determination values (R<sup>2</sup>) ranging from 0.83 to 0.97 and 0.72 to 0.86, capturing the variability observed in the outputs of the 'Beaumont' and '788' cultivars, respectively. Notably, the prediction of high L\* values: 69,32 for '788' and 79,42 for 'Beaumont' occurred under minimal drying duration and high storage temperature. Validation experiments supported these predictions, yielding L\* values of 79.75 for 'Beaumont' and

76.25 for the '788' varieties, which fall within the recommended L\*. The firmness model exhibited significance ( $P < 0.0001$ ) specifically for the '788' cultivar. These findings show the efficacy of process modelling and optimization in enhancing the quality of macadamia nuts. However, further investigations are warranted, particularly concerning the long-term storage and shelf life, to comprehensively address the biochemical parameters of macadamia nuts and maximize overall quality.

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## CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

### 7.1 Conclusions

The macadamia nut industry constantly faces the challenge of inconsistent abscission seasons, resulting in variable harvest times and increased operational costs. To address this, ethephon is widely employed to induce uniform nut abscission and streamline harvesting. However, understanding how ethephon affects the nutritional quality and postharvest shelf life is crucial. This study investigates the influence of ethephon on the postharvest quality of two macadamia nut cultivars, '788' and 'Beaumont.' Specifically, it examines the impact on fatty acids and dietary indicators, assesses variations in biochemical composition among nuts harvested using different methods, and evaluates the effects of preharvest ethephon on the physical attributes of roasted macadamia nuts. Additionally, the research explores the impact of storage conditions on the firmness and colour attributes of ethephon-harvested nuts, utilizing Response Surface Methodology.

#### 7.1.1 Contributions to knowledge

Firstly, the study significantly advanced our understanding of how ethephon, a key plant growth regulator, affects macadamia nuts, with implications for fatty acid composition and dietary indicators. Notably, the 'Beaumont' cultivar exhibits increased saturated fatty acids (SFAs) and decreased unsaturated fatty acids. Thus, hinting at the potential of ethephon treatment methods' modification and careful cultivar selection as a good strategy to optimize the nutritional value and shelf-life of macadamia nuts.

Furthermore, the study reveals cardiovascular implications associated with ethephon, particularly in the 'Beaumont' cultivar, as evidenced by changes in atherogenic and thrombogenic indices. This highlights the importance of considering cardiovascular risks in

the consumption of ethephon-treated macadamia nuts. Additionally, the cultivar-specific nature of this response reveals that postharvest practices may elicit unique cultivar responses rather than generalized outcomes in macadamia nut production.

Also, the study uncovers intricate connections among biochemical parameters, polyphenol oxidase activity, and various storage methods in macadamia nut production. A strong positive correlation was observed between sucrose content and polyphenol oxidase activity in the 'Beaumont' cultivar. This sheds light on the complex relationships influencing overall quality and oxidative processes in macadamia nuts.

Investigating the factors influencing the physical characteristics of '788' and 'Beaumont' cultivars, the study further highlights variations in nut mass, diameter, colour attributes, and texture under diverse treatments and storage conditions. This comprehensive insight is essential for understanding how environmental and treatment factors collectively shape the physical attributes of macadamia nuts.

Finally, the application of Response Surface Methodology (RSM) to optimize postharvest parameters and colour-related values enhances study precision and provides a robust framework for industry practitioners seeking to maximize the quality of macadamia nuts. Achieving high coefficients of determination and successfully predicting optimal values for parameters such as  $L^*$ ,  $a^*$ ,  $b^*$ , and Hue angle reveal the effectiveness of process modelling and optimization of macadamia nuts parameters for optimal quality.

In summary, this research addresses critical knowledge gaps, providing detailed insights into ethephon's effects on various facets of macadamia nut quality. By unraveling complexities, identifying correlations, and offering practical optimization methodologies, the study lays a foundation for informed decision-making in both academic and industrial contexts. These contributions position this research as a pivotal milestone in advancing macadamia nut production and postharvest management.

## **7.2 Recommendations**

While this study provides valuable insights, it also acknowledges certain challenges and areas for further exploration. This study highlights the need for a holistic understanding of the nutritional implications of ethephon treatment on macadamia nuts. While the analysis of fatty acid composition and dietary indicators provides crucial biochemical insights, the study acknowledges the imperative of incorporating comprehensive sensory evaluations in future studies. Such evaluations would offer a more complete perspective on how these observed changes may impact the overall quality and consumer acceptability of macadamia nuts. By bridging this sensory gap, future research can provide valuable insights into the practical implications of the biochemical alterations observed in this study.

The identification of an increase in thrombogenic indices in the ‘Beaumont’ cultivar raises concerns regarding potential cardiovascular risks associated with ethephon application. This significant finding calls for further research to investigate the specific mechanisms underlying these cardiovascular implications. Furthermore, while the biochemical findings of this study favours the labour-intensive harvesting method (CT), where nuts are manually picked directly from the tree, in contrast to the relative ease of ethephon application, it is important to carefully weigh the benefits against practical considerations such as labour intensity and detailed associated costs. Nevertheless, future research could explore strategies to optimize harvesting methods, potentially integrating mechanized approaches to reduce labour requirements without compromising the quality of the harvested nuts. This consideration is vital for ensuring the scalability and economic viability of macadamia nut production.

### **7.2.1 Future possibilities**

The study's findings unlock promising avenues for future research and opportunities in the realm of macadamia nut production: Firstly, there is potential for delving into refined

ethephon application strategies that aim to minimize adverse effects on fatty acid composition. Investigating nuanced approaches to the application of ethephon could provide insights into preserving the nutritional quality of macadamia nuts while still benefiting from its ripening effects. Secondly, the identification of cultivar-specific responses suggests the possibility of selecting less sensitive macadamia cultivars. By carefully choosing cultivars that exhibit resilience to changes in fatty acid profiles, producers can work towards maintaining a more desirable composition in the final product, thus enhancing overall product quality. Thirdly, the study suggests the importance of optimizing biochemical parameters and minimizing polyphenol oxidase activity in ethephon-treated nuts for improved quality during storage. Further research could focus on the use of advanced modelling techniques such as application of the use of machine learning algorithms. Lastly, there is a call for long-term studies that specifically concentrate on storage conditions and shelf-life considerations. Investigating the extended effects of storage conditions on macadamia nuts and developing protocols for optimal shelf life could significantly contribute to the refinement of postharvest practices in the macadamia industry.

### **7.3 Final comments**

In conclusion, this comprehensive study thoroughly examines the complex impacts of ethephon on ‘788’ and ‘Beaumont’ macadamia nuts. A key takeaway is the recognition of the imperative for tailored post-harvest interventions, acknowledging and accommodating the distinct responses exhibited by each cultivar. This research not only contributes significantly to the academic understanding of macadamia nuts but also provides invaluable practical insights for professionals in the industry. The evolving landscape of the macadamia sector demands well-informed decision-making, and the insights garnered from this study emerge as a critical resource. As the macadamia industry continues to evolve, the knowledge distilled

from this research stands as a foundation for industry leaders, guiding them in making informed decisions that not only optimize production practices but also maintain the quality of macadamia nuts in the dynamic global market.