Effect of short-term gaseous ozone treatment on postharvest quality and shelf-life of ‘Eureka’ lemons

By

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Declaration

I, **Hlumelo Mhlwatika**, 222049202, affirm that this dissertation represents my own original research, with all external sources properly cited. The content, including data, visuals, and analysis, is my own work unless explicitly attributed to others. This thesis is unique to this submission and has not been used for any other academic qualification at any institution. The research data has not been presented elsewhere for degree purposes.

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General abstract

Citrus is globally one of the commonly consumed fruits, its consumption has steadily increased over the last decades. It has a significant contribution to the South African economy and employment. The 2024 report by Citrus Growers' Association (CGA), indicated that South Africa exported 164.5 million 15kg cartons of citrus, with lemons contributing 21% of exports. Similar to other fresh produce, 'Eureka' lemons are highly sensitive during postharvest handling and their quality tends to deteriorate. The postharvest treatments are necessary to minimize the quality loss and extend the shelf-life.

Gaseous ozone (O_3) is an emerging postharvest treatment for preserving the postharvest quality and extending the shelf-life of fresh horticultural produce. Therefore, this study explored the effect of the short-term gaseous ozone on the quality of 'Eureka' lemons. The study aimed to: 1) investigate the effect of gaseous ozone on the physical attributes on the quality and shelf-life (Chapter 3); 2) assess the effect of gaseous ozone in antioxidant and biochemical attributes (Chapter 4); and lastly, evaluate the impact of gaseous ozone on senescence-related enzymes of 'Eureka' lemons during cold storage (Chapter 5).

The first experiment investigated the effect of gaseous ozone on the physical quality attributes such as decay, mass loss, colour and firmness. The findings revealed O_3(48h) caused severe decay, this treatment showed high decay incidence as compared to the O_3(24h) and the untreated lemons. However, for mass loss, firmness and colour, the O_3(48h) treatment showed positive results as there was a relatively lower loss compared to the other treatments.

The second experiment revealed that ozone treatment duration has a significant impact on the biochemical attributes of the fruit. For instance, O_3(24h) treatment positively affected the phenolics, flavonoids and DPPH. However, O_3(48h) showed a positive influence on the carotenoids, ascorbic acid and condensed tannins. This shows that biochemical parameters exhibit unique responses to the oxidizing power of ozone.

The third objective assessed the impact of gaseous ozone on senescence-related enzymes such as catalase, peroxidase, lipoxygenase and glutathione reductase as well as lipid peroxidation. This experiment revealed that O_3(24h) is effective in preserving the senescence-related enzymes as there was an increase from Day 0 to Day 35 in all the enzymes. On the other hand, the O_3(48h) treatment at ambient storage showed a decreasing trend in catalase, lipoxygenase

and glutathione reductase. Ozonation inhibits the accumulation of O_2 and H_2O_2 which is associated with the higher activity of the peroxidase.

Based on these findings, O_3(48h) is less likely to be recommended as it carries high oxidizing power which adversely affects the quality of the fruit. The O_3(24h) revealed the possibility of maintaining the quality without severely affecting the fruit. There is however a need to further optimize the ozone treatment protocols, for instance, the performance of O_3(36h), which is slightly lower than the 48h, should be assessed. Also, the combination of gaseous ozone and the edible coatings should be explored.

Preface

This dissertation is a collection of individual manuscripts, each chapter functioning as an independent entity, which has led to some unavoidable repetition between chapters.

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CHAPTER 1

General Introduction

1.1. Introduction

Citrus is one of the commonly consumed fruits owing to its high nutritional attributes and health benefits (Kundu et al., 2019). It contains bioactive compounds such as flavonoids, limonoids, phenolic acids, vitamin C and volatile terpenoids that are essential for diseases prevention (Kundu et al., 2019). Some of the phytochemicals in citrus fruit have been demonstrated to possess anticarcinogenic, anti-inflammatory, antiallergic, antimicrobial and antidepressant properties (Abobatta, 2019). The increased intake of citrus fruit, such as lemons, has been strongly linked to reduced risk of cardiovascular diseases, blood pressure, respiratory problems, kidney stones and arthritis (Qudah et al., 2018).

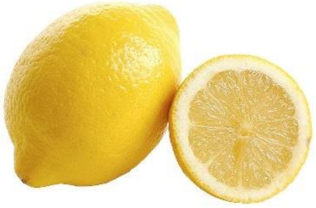
Citrus is categorised as the non-climacteric fruit (Sonkar et al., 2008), and if the conditions that the fruit is exposed to after harvesting are not controlled, its quality quickly deteriorates. Postharvest disorders and diseases play a major role in the deterioration of the fruit (Strano et al., 2017). The major problem that citrus growers are faced with is the deterioration of quality in terms of the appearance and internal quality (Strano et al., 2017). Consumers purchase fruit based on visual instinct (Alam, 2020), this is particularly important for fruit destined for the export market. If the fruit is non-compliant in terms of quality standards of the Perishable Products Exports Control Board (PPECB) it is likely to be rejected during quality inspections at packhouses and depots (Cronje et al., 2017).

Chilling injury is one of the leading causes of postharvest losses in citrus fruit (Belwal et al., 2020). The chilling injury symptoms include rind pitting, poor colour development, unpleasant taste, and increased susceptibility to microbes (De Ell, 2004). Fruit is at higher risk of reaching senescence earlier than expected when it is exposed to warm conditions after harvesting. High temperatures increase the risk of bacteria infestation in and on the fruit. The study conducted by Membré et al. (2004), demonstrated that most bacteria are thermophilic and strive well in warm conditions. The economic important diseases in citrus fruit include green mould, blue mould and sour rot, which are caused by *Penicillum digitatum* (Persoon), *Penicillum italicum* (Wehmer) and *Geotrichum candidum* (Link) respectively (Strano et al., 2017). On the other hand, postharvest disorders such as oleocellosis, rind pitting and decay also make a significant contribution to fruit loss (Özdemir, 2021).

The use of postharvest treatments for reducing postharvest stress and extending the shelf-life of fresh produce has become common in recent years. Nitric oxide (NO) is one of the chemical postharvest treatments used to preserve the quality of the fresh produce. NO is highly reactive and it acts as a multifunctional signalling molecule in various plant physiological processes, such as fruit ripening and senescence of fresh fruit (Wendehenne et al., 2004). Optimum NO application prolongs the postharvest storage life by delaying ripening and senescence, suppress biosynthesis of ethylene, and reduce ethylene production (Singh et al., 2013). Ren et al. (2004) suggests that the reduction in ethylene production during fruit ripening in NO-fumigated fruits may be due to the inhibition of 1-aminocyclopropane-1-carboxylicacid (ACC) synthase and ACC oxidase. There are health risks which are associated with the use of NO, which makes it unsafe to some extent. The health risks include, nose and throat irritation, build-up of fluid in lungs, inability to transport oxygen by the blood (New Jersey Health, 2009).

Sulfur dioxide (SO_2) is used to prevent decay during storage by initial fumigation of the fruit from the field followed by the weekly fumigation of the fruit (Palou et al., 2001). Lemon (Fig. 1.1) is one of the fruits that have been tested for the efficacy of SO_2 (Sivakumar et al., 2010). According to the report by Cantin et al. (2012), fumigation of SO_2 followed by controlled atmosphere storage, is found to be effective against decay in blueberries and maintain high nutritional value. There are advantages that are associated with the use of SO_2; the concentration applied may induce injuries in grapes and sulfite residues on the fruit may be risky for some individuals, cause firming of the texture of some fruit species, for instance pomaces (Cantin et al., 2012).

Heat treatment (HT) is one of the commonly used postharvest treatment in citrus fruit. HT has been applauded by a lot of commercial growers, due to the fact it does not leave residues on the fruit surface. Reduced fruit decay and disease incidence have been reported in heat treated citrus fruit (Strano et al., 2022; Yun et al., 2013). However, like many other postharvest interventions, HT also pose some challenges. For instance, if the fruit is subjected to hotter temperatures, the quality can deteriorate (Wan et al., 2020). Signs of deterioration include browning (Fig. 1.3), which is a loss of green colour (Kaewsuksaeng et al., 2015). Browning later progresses to decay, which in advanced stages is identified as the sour rot (Fig. 1.2) caused by *Geotrichum candidum* and affects the edibility of the fruit (Strano et al., 2017).



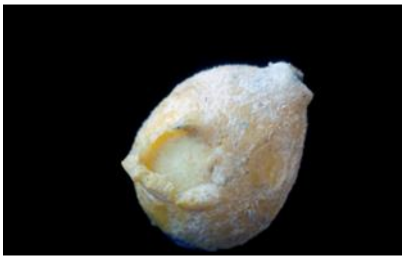


Figure 1.1: Lemon in good quality Figure 1.2: Lemon with sour rot

Irradiation is one of the effective methods for improving postharvest quality of the fresh produce. Irradiation can be applied to the produce by ionizing radiations, such as gamma rays (60Co or 137Cs), electron beams (β particles) and X-rays, or by non-ionizing radiations, such as ultraviolet light in any packaging material (Komolprasert, Packaging food for radiation processing, 2016). Current and potential applications of ionizing radiation in postharvest handling of fresh horticultural perishables have shown to delay decay, slow senescence and deactivate pathogen growth on fruit surface (Kader, 1999). However, according to Sharma et al. (2020), higher dosage of irradiation causes browning on fresh produce (Fig. 1.3).

Figure 1.3:Browning on lemon

Ozone is one of the emerging technologies that has shown potential as a postharvest treatment. Bocci (2005) defined ozone as a triatomic inorganic molecule that is made up of three atoms of oxygen. Ozone is a highly unstable matter that, under specific conditions, such as pressure and temperature, splits into oxygen atoms with a short life span, which is why after a certain period ozone will decay into its original form. Ozone is highly oxidative and is used as an antimicrobial agent against numerous species of different microorganisms (Panou et al., 2021).

Ozone eliminates bacteria by attacking the molecular structure of their protective membranes and altering the internal enzymes (Remondino & Valdenassi, 2018). Ozone has been noted to be effective within short spaces of time. According to the study conducted by Das et al. (2006), ozone gas at 10 mg/L resulted in a ~7 log10 CFU/tomato reduction of *Salmonella enteritidis* inoculated on tomatoes within 1 h. Concha-Meyer et al. (2014) followed by a study on blueberries where gaseous ozone at 4 mg/L reduced *Listeria monocytogenes* by 3 log10 CFU/ml on fresh blueberries after 10-day storage at 4℃. According to the study conducted by Murray et al. (2018), a 20-min gaseous ozone (23 mg/L) exposure caused 2.1–3.1 log10 CFU/apple reduction of *L. monocytogenes* on 'Empire' apples.

Ozone can be applied in aqueous or gaseous form (Panou et al., 2021). Out of these two, the gaseous ozone has been found to be the most effective because it is not mixed with any compound while aqueous ozone can be found ineffective due to the poor quality of water used (Shezi et al., 2020). Shezi et al. (2020) further highlights that ozone efficacy is affected by various factors which include the type of produce treated, stage of development (age) of a produce, duration of treatment, method of treatment, namely, continuous or intermittent, storage temperature and relative humidity. The advantageous use of ozone is that after the treatment on the fruit, there are no residues left on the surface (Panou et al., 2021). The germicidal effect of ozone is based on its oxidative effect on cell membranes of microorganisms (Cataldo, 2003). Physiologically, gaseous ozone regulates the rate at which ethylene is produced (Fan, 2021). The rate at which the ethylene is produced determines the rate at which senescence will be reached. This implies that, ozone is essential for delaying fruit ageing.

1.2. Research rationale

After harvesting, 'Eureka' lemons start to deteriorate in quality. Sudden metabolic changes occur resulting to early senescence, which predominantly occurs when the fruit is stored in uncontrolled atmospheric conditions (Strano et al., 2022). Such metabolic changes include high respiration rate, transpiration rate, and high ethylene production fruit (Lafuente & Zacarias, 2006). These metabolic changes are precursors of physiological disorders which become visible on the surface of the fruit (Lafuente and Zacarias, 2006). The most common disorders include oleocellosis, stem-end rind breakdown, stylar-end breakdown, blossom-end clearing, and chalking which is caused by condensation of water wax on the fruit surface (Lafuente and Zacarias, 2006).

Overripening is one of the factors affecting the quality of lemons. Overripening is associated with rapid senescence due to higher ethylene accumulation during storage (Satekge &

Magwaza, 2022). With the issues surrounding lemons, there are vast of methods that are currently used to prolong the shelf life of the fruit and delay senescence.

Currently, chlorine and chlorine dioxide are some of the treatments used in the citrus industry applied on fruits, in which Feliziani et al. (2016), indicate that even when applied at the higher concentration, it cannot control the pathogens which are in wounds. That creates opportunity for disease advancement due to the live pathogens in the fruit. Modified and controlled atmospheric storage is also used to extend the shelf-life of citrus fruit by modifying the storage atmospheric conditions by creating medium to low carbon dioxide which reduces respiration and fungal growth (Baswal et al., 2020). There are limitations associated with the use of modified atmospheric packaging which include, capital cost of machines, packaging materials, calculation costs of the desired gas are high and in some cases off-flavours develop, there is a possibility of food-borne pathogens development (Khan & Mittal, 2020). The disadvantages listed indicate that there is more research that still needs to be done on the efficacy of the treatments used to preserve postharvest quality of the fresh produce and thus delay senescence.

For quite some time, ozone has been used to preserve the quality of various horticultural fresh produce. For instance, Previdi et al. (2016) showed that 1 ppm of gaseous ozone gas eliminated *L.innocua* in blueberries. Similarly, Yaseen et al. (2015) observed that ozone treatments suppressed *P. expansum* activity on apples fruits of varieties Golden Delicious, Royal Gala and Fuji in the storage of 30, 45 and 60 days. These researchers further elaborated that fruit stored under ozone had higher concentrations of glucanase, peroxidase and phenylalanine ammonia-lyase. Glucanase is well-known biochemical marker of systemic acquired response (SAR). An increase in glucanase provides resistance against pathogens (Yaseen et al., 2015). Peroxidase helps to protect plants against oxidative damage reducing free radical production from hydrogen peroxide (Nnamchi et al., 2021).

There is currently no data on how gaseous ozone affects the quality of ‘Eureka’ lemons. Palou et al. (2001) assessed the effect of ozone on the development of green and blue mould in oranges and lemons. It was discovered that ozone managed to slow down ethylene production, reduce diseases incidence by tampering with the influence of microbes on fruits. However, the effect of gaseous ozone on ‘Eureka’ lemons is yet to be assessed. This is particularly important for the South African industry as it is consistently searching for novel and non-chemical postharvest treatments in order to maintain its global standing as the leading exporter of superior quality ‘Eureka’ lemons.

Among all the fruits, citrus fruits and juice have a great reputation for a large number of people as a nutritious food. The presence of vitamin C content in the fruits makes it more popular among the community (Kundu et al., 2019; Singh et al., 2020). Recently people are more concerned about bioactive compounds, which include vitamins, flavonoids, limonoids, phenolic acids, and volatile terpenoids that prevent different diseases. Citrus fruits contain all the constituents mentioned above which help to maintain good health (Berk, 2016).

1.3. Aim

The study aims to investigate the potential of short-term gaseous ozone treatment in preserving the quality and delaying senescence of 'Eureka' lemons.

1.4. Objectives

The specific objectives of the study are to:

- Investigate the effect of gaseous ozone on the physical attributes on the quality and shelf-life of the 'Eureka' lemons

  Parameters to be measured include decay, mass loss, colour and firmness.
- Assess the effect of gaseous ozone in antioxidant and biochemical attributes of 'Eureka' lemons.

  Parameters to be measured include total phenolics, flavonoids, total carotenoids, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid.
- Assess the impact of gaseous ozone on senescence-related enzymes of 'Eureka' lemons during cold storage.

  Parameters to be measured include catalase, peroxidase, lipoxygenase and glutathione reductase as well as lipid peroxidation.

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CHAPTER 2

Recent advances on postharvest treatments of citrus fruit: A review

Abstract

Citrus fruits are prone to various physiological disorders, including chilling injury and rind breakdown, as well as pathological issues such as brown rot, sour rot, green mold, and blue mold. Consequently, effective postharvest treatments are essential for preserving and extending the shelf-life of these commodities. The citrus industry predominantly relies on chemical treatments that have demonstrated high efficacy against postharvest pathogens and disorders. However, increasing evidence suggests that many synthetic chemicals may pose health risks to humans and the environment. In response to these concerns, recent research in postharvest technology has shifted towards developing non-chemical and environmentally friendly treatments for fresh horticultural produce. Among these alternatives, edible coatings and gaseous ozone have emerged as promising options for postharvest applications. This review aims to elucidate various postharvest treatments utilized in citrus fruit preservation and their impact on fruit quality. Nonetheless, there remains a paucity of literature regarding the effects of certain treatments, such as gaseous ozone, particularly concerning its influence on 'Eureka' lemons.

Keywords: Postharvest technology; quality; postharvest disorders; citrus; chemical treatments; non-chemical treatments; shelf-life.

2.1 Introduction

Citrus fruits are widely recognized for their quality and associated health benefits (Kundu et al., 2019). Their physical and chemical characteristics contribute significantly to their market preference over other fruits. Key physical attributes encompass colour, fruit mass, firmness, and diameter, which includes both polar and equatorial measurements (Kassim et al., 2020). The primary chemical characteristics include total soluble solids (TSS), pH, acid ratio, as well as concentrations of ascorbic acid and citric acid (Al-Saif et al., 2022). The consumption of citrus fruits is linked to their health benefits, including a reduction in the risk of diseases such as hypertension and diabetes (Lv et al., 2015; Kakutani et al., 2022). However, significant postharvest losses pose a substantial challenge to the production and availability of citrus fruits.

Postharvest losses in citrus fruits, attributed to both physiological and pathological disorders, account for approximately 30-50% of production losses (Strano et al., 2022). Inadequate postharvest handling increases the susceptibility of fruits to various physiological and pathological disorders (Kassim et al., 2020; Kalatippi & Hota, 2021). Among the economically significant physiological disorders affecting citrus fruits are chilling injury, creasing, stem-end rind breakdown, stylar end breakdown, and oleocellosis (Kalatippi & Hota, 2021). The pathological disorders, such as green and blue mold, can significantly accelerate fruit deterioration (Altieri et al., 2013). This decline in quality ultimately results in financial losses, as consumer purchasing decisions are often driven by visual appeal (Tesfay & Magwaza, 2017; Alam, 2020).

The preservation of citrus fruit shelf-life is a critical aspect of postharvest management, with fungicide application in packhouses identified as a most commonly used strategy (Altieri et al., 2013). However, improper application of these fungicides poses significant risks. Specifically, the application of imazalil, a commonly used fungicide, in conjunction with wax coatings can result in residual deposits on the surface of the fruit (Altieri et al., 2013).

Furthermore, research has demonstrated that excessive use of fungicides can lead to the development of pathogen resistance. This resistance not only diminishes the efficacy of these treatments but may also facilitate the transfer of genetic traits associated with resistance to humans, potentially leading to adverse health effects (Tahiri et al., 2022). Thus, while fungicides are essential for managing postharvest diseases, their application must be carefully regulated to mitigate risks to both fruit quality and human health.

Chemical treatments such as 1-methylcyclopropene (1-MCP) and salicylic acid (SA) have been demonstrated to effectively preserve the quality of citrus fruits. In a study conducted by Pristijono et al. (2018), it was observed that 'Tahitian' limes treated with 0.5 μL/L of 1-MCP exhibited diminished ethylene production, reduced calyx abscission, and maintained peel greenness throughout storage. Concurrently, the application of salicylic acid has been shown to enhance the activity of various disease resistance-related enzymes, including β-1,3-glucanase, phenylalanine ammonia-lyase, and peroxidase. This enhancement is attributed to the induction of pathogenesis-related gene expression, thereby bolstering disease resistance in plants (Zhu et al., 2016). Nonetheless, it is crucial to note that the postharvest application of chemical treatments poses potential risks not only to environmental health but also to human safety (Bambalele et al., 2021).

Various techniques and treatments have been developed and studied to maintain the quality of fruits and reduce postharvest losses. Among these, edible coatings and gaseous ozone have garnered considerable attention from researchers. According to Bambalele et al. (2021), edible coatings are derived from natural sources and often incorporate antioxidants and vitamins that are advantageous for human health. These coatings function by reducing transpiration and respiration rates, thereby minimizing the loss of water and solutes, which subsequently leads to a decrease in microbial activity on the fruit (Ncama et al., 2018).

The application of gaseous ozone is recognized for its antimicrobial properties and ability to enhance the shelf-life of fresh produce. Ozone treatment can effectively inhibit the growth of pathogens while preserving the sensory and nutritional quality of fruits. The synergistic effect of combining gaseous ozone with edible coatings has been shown to further improve the postharvest quality by maintaining firmness, delaying ripening, and enhancing antioxidant activity in fruits such as mangoes (Bambalele et al., 2021; Ncama et al., 2018).

Heat treatments, including hot water and hot air applications, have been documented as effective methods for prolonging the shelf-life of citrus fruits (Palou, 2013). The efficacy of these thermal treatments is influenced by several factors, including the specific cultivar, the initial condition of the fruit prior to treatment, the temperature and duration of the heat application, the method of heat delivery, and the type of pathogens present (Palou, 2013).

However, the effectiveness of heat treatment is not universally guaranteed. For instance, a study conducted by Yun et al. (2013) found no significant differences in respiration rates or soluble solute concentrations between ‘Satsuma’ mandarins subjected to a hot water dip at 52 °C for 2 minutes and untreated control fruits. These treated fruits were subsequently stored in a ventilated environment at temperatures ranging from 12 to 16 °C with relative humidity levels between 90-95% for a duration of 60 days. This highlights the necessity for developing treatments that are both effective in enhancing fruit longevity and environmentally sustainable.

Studies and reviews have been done on how various postharvest treatments affect the quality of horticultural products (Table 2.1). There is still however a need to conduct more research on how the quality of the fresh produce is affected by various postharvest treatments. For instance, there are no studies on how ‘Eureka’ lemon is affected by the gaseous ozone. Furthermore, currently there are no reviews on citrus postharvest treatments. This review provides guidance on the work that has been done and how the fruits are affected by postharvest treatments.

Table 2.1: Previously published review articles on postharvest treatments of horticultural products

Review	Fresh produce	Reference
Yeast Warriors: Exploring the Potential of Yeasts for Sustainable Citrus Post-Harvest Disease Management	Citrus	Ezzouggari et al. (2024)
Postharvest Technologies of Fresh Citrus Fruit: Advances and Recent Developments for the Loss Reduction during Handling and Storage	Citrus	Strano et al. (2018)
Recent Developments on Dynamic Controlled Atmosphere Storage of Apples—A review	Apples	Mditshwa et al. (2018)
Postharvest Handling Practices and Treatment Methods for Tomato Handlers in Developing Countries: A Mini Review	Tomato	(Arah, Ahorbo, Anku, Kumah, & Amaglo, 2016)
A Review of the Postharvest Characteristics and Pre-packaging Treatments of Citrus Fruit	Citrus	Kassim et al. (2020)
Biological Control of Citrus Postharvest Phytopathogens	Citrus	Moraes-Bazioli et al. (2019)
Essential Oils Amended Coatings in Citrus Po stharvest Management	Citrus	Bhandari et al. (2018)

2.2 Citrus quality attributes

In fresh fruit, quality is a term that may convey multiple interpretations. Within the fresh produce markets, quality often refers to the external appearance of the product (Abouzari & Nezhad, 2016). However, according to Lado et al. (2018), fruit quality does not only refer to external parameters only but also includes internal attributes. The quality of the fruit is the deciding factor on purchasing the product. Thus, it is important for the fruit to possess good physical and chemical attributes. The section below highlights some of the key quality attributes of citrus fruit.

2.2.1 Physical attributes

2.2.2.1 Skin colour

Fruit colour is a critical attribute influencing consumer perception and product acceptability, as it is often the first characteristic noticed by consumers (Abouzari & Nezhad, 2016; Kassima, 2013). The observed colour changes during fruit ripening are associated with several underlying processes: Chemical mechanisms (these involve the breakdown of cellular structures such as chloroplasts and chromoplasts, which play a significant role in pigment transformation); biochemical changes (the transformation and degradation of natural pigments, including chlorophylls, carotenoids, and anthocyanins, contribute to the dynamic colour shifts observed in ripening fruits), and physicochemical mechanisms (these encompass various physical and chemical changes that affect the fruit's appearance and texture during the ripening process (Sinha et al., 2012).

2.2.2.2 Fruit size

Size is a critical factor in the marketing of fruit, particularly for small, easy-peeling cultivars such as 'Clementine' (Abouzari & Nezhad, 2016). Research indicates that fruits exceeding 70 mm in diameter are predominantly favoured by consumers and command higher market prices (Abouzari & Nezhad, 2016). Balaguera-Lópe et al. (2020) emphasize that the presence of seeds significantly influences fruit size, noting that seedless varieties tend to be smaller than their seeded counterparts. The process of fruit development is essential to understanding fruit size, as it encompasses a complex interplay of biological mechanisms related to cell division and expansion (Ferrer et al., 2017; Renaudin et al., 2017).

2.3 Chemical attributes

2.3.1 Total Soluble Solids (TSS) and Titratable acidity

Total soluble solids (TSS) significantly influence fruit quality characteristics (Magwaza et al., 2012). TSS comprises 10-20% of fruit mass, with carbohydrates constituting 70-80% of these solids (Kassim et al., 2016). As fruits mature, TSS content rises, serving as a maturity indicator for harvest timing (Farag et al., 2020).

The composition of TSS includes 80% sugars (predominantly fructose, glucose, and sucrose), 10% acids (citric, malic, and oxalic), and 10% nitrogenous compounds (Seminara et al., 2023). Through fruit development, sugar levels increase while organic acids and citric acid decrease.

Table 2.2: Comparison of the metabolites content in the pulp of different citrus varieties

Metabolite	Lemon	Orange	Grapefruit	Mandarin
Sugars (Mg/g FW)	90-100	100-130	60-120	110-140
Fructose (Mg/g FW)	10-30	20-50	20-30	10-30
Glucose (Mg/g FW)	10-20	20-50	10-30	10-40
Sucrose (Mg/g FW)	5-10	50-60	30-70	60-80
Malic acid (g/L)	1-3	1-3	0.3-2.0	0.2-2
Citric acid (g/L)	40-45	6-12	4-9/13-27	8-12
Phenolic acid (Mg/g FW)	49,8	0.90-1.0	60-72	0.50-0.85
Ascorbic acid (Mg/g FW)	0.30-0.60	0.40-0.50	0.30-0.35/0.30-1.25	0.20

(Aschoff et al., 2015; Lado et al., 2016; Emmanouilidou & Kyriacou, 2017; Elkhatim et al., 2018).

2.3.2 Phenolics

Phenolic compounds, commonly referred to as phenolics, represent a diverse class of organic compounds characterized by the presence of one or more hydroxyl groups (−OH) directly attached to an aromatic hydrocarbon ring (Fig 2.1). The specific phenolic acid profiles can vary significantly among different cultivars (Table 2.2).

Research indicates that phenolic acids play a crucial role in the antioxidant capacity of *Citrus sinensis* (sweet oranges) (Seminara et al., 2023). A study conducted by Emmanouilidou and Kyriacou (2017) on the cultivars 'Lane Late' and 'Delta' revealed that phenolic concentrations peaked at 363 mg/L and 413 mg/L, respectively, before declining to 330 mg/L and 360 mg/L one month later. This suggests that mature fruits typically exhibit lower phenolic acid levels compared to their immature counterparts.

Additionally, it has been observed that phenolic acid content is significantly higher in the pulp than in the rind of the fruit. The levels of these compounds decrease during fruit maturation but can increase during postharvest cold storage, indicating a dynamic response of phenolic compounds to both developmental and storage conditions (Quin et al., 2014). This fluctuation in phenolic content underscores the importance of phenolics in fruit quality and their potential health benefits.

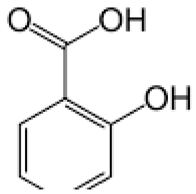

Figure 2.4: Chemical structure of phenolic acid

2.3.3 Vitamins

The flesh of citrus fruits serves as a significant source of vitamins, with vitamin C, also known as ascorbic acid, being the most prominent due to its antioxidant properties and various nutritional benefits (Najwa & Azlan, 2017; Goldenberg et al., 2014; Zou et al., 2015). Vitamin C is comprised of ascorbic acid and its oxidized form, dehydroascorbic acid (Lado et al., 2018), characterized by the structural representation shown in Figure 2.2.

In addition to vitamin C, citrus fruits contain other essential vitamins, including folate (as reduced 5-methyl tetrahydrofolate and polyglutamate derivatives), pro-vitamin A compounds such as β-carotene and β-cryptoxanthin, and vitamin E (Zou et al., 2015). Vitamin C plays a crucial role in human health by supporting the maintenance of connective tissues (Aghajanian et al., 2015) and is involved in the biosynthesis of vitamin B and folic acid, as well as in the conversion of cholesterol to bile acids (Chambial et al., 2013). Its antioxidant properties are vital for protecting cells from oxidative stress (Seminara et al., 2023), with ascorbic acid effectively safeguarding cellular components from damage caused by free radicals (Barańska, 2020).

Quantitative analyses indicate that vitamin C levels are generally higher in oranges compared to grapefruit and mandarins (Table 2.2) (Alos et al., 2014). Notably, Alos et al. (2014) also reported that ascorbic acid concentrations tend to be greater in the rind than in the pulp, typically increasing during fruit maturation but declining in the pulp.

Several mechanisms and biosynthetic pathways have been proposed to regulate ascorbic acid levels in the peel and pulp of citrus fruits. The L-galactose pathway is primarily active in the peel, where selective stimulation of GDP-mannose pyrophosphorylase (GMP) and GDP-L-galactose phosphorylase (GGP) promotes a higher flow of metabolic precursors during fruit ripening in oranges compared to mandarins (Lado et al., 2018; Alos et al., 2014). Additionally, the D-galacturonic acid pathway, particularly involving the GalUR12 gene, may also play a role in regulating ascorbic acid concentrations within the flavedo of citrus fruits (Lado et al., 2015a).

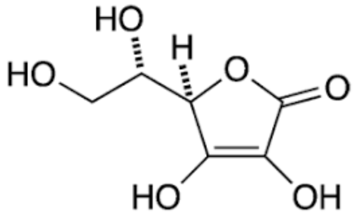

Figure 2.5: Chemical structure of ascorbic acid

2.3.4 Ethylene

Citrus fruits are classified as non-climacteric fruits; however, they can also be described as pseudoclimacteric due to their responsiveness to exogenous ethylene (Alos et al., 2014). Ethylene biosynthesis in citrus is initially regulated by an autocatalytic-like system during early fruit development, transitioning to an auto-inhibitory system upon maturation (Alos et al., 2014; Lado et al., 2018).

The primary function of ethylene in citrus fruits is to facilitate degreening by promoting the degradation of chlorophyll and enhancing the development of carotenoids (Lado et al., 2018). Ethylene exerts its effects by inhibiting the expression of magnesium chelatase, an enzyme crucial for photosynthesis and chloroplast biosynthesis (Zhang et al., 2018). Additionally, ethylene stimulates the transcription of carotenoid biosynthetic genes in the peel, facilitating the conversion of chloroplasts to chromoplasts and leading to the accumulation of xanthophylls and apocarotenoids in orange and mandarin peels (Rodrigo et al., 2013a, 2013b).

Research conducted by Mayuoni et al. (2011a) indicates that ethylene does not significantly affect internal parameters such as juice content, acidity, and total soluble solids (TSS) in certain mandarin varieties. However, it has been shown that ethylene is involved in transcriptomic changes within the pulp of mandarins, activating various metabolic pathways related to secondary metabolism, gibberellin activity, auxin degradation, and responses to biotic and abiotic stresses (Mayuoni et al., 2011b). This highlights the multifaceted role of ethylene in both the physiological and biochemical processes occurring in citrus fruits during maturation and ripening.

2.4 Postharvest storage conditions affecting citrus fruit quality

Postharvest storage conditions significantly influence the quality and shelf-life of citrus fruits. Key factors affecting the postharvest quality of citrus include storage temperatures, humidity levels, handling practices, and packaging methods. Inadequate storage conditions can lead to physiological disorders that accelerate the onset of senescence, causing the fruit to deteriorate more rapidly than anticipated.

2.4.1 Temperature

Temperature is a critical factor in maintaining the quality of citrus fruits during postharvest storage, necessitating constant monitoring to optimize conditions. According to Ladanyia & Ladanyia (2010), low storage temperatures effectively reduce metabolic rates and inhibit fungal development, thereby preserving the quality of the fruit and extending its shelf-life. Research by Qiu and Wang (2015) demonstrated that 'Satsuma' mandarins stored at 4°C exhibited significantly higher vitamin C levels compared to those stored at 20°C, underscoring the importance of understanding the optimal storage temperatures for different citrus varieties. Additionally, Mditshwa et al. (2017) highlighted that under ambient conditions, citrus fruits experience a more rapid decline in quality, further emphasizing the necessity for controlled storage environments to sustain freshness and nutritional value.

2.4.2 Gas composition

The use of controlled atmosphere (CA) has been proven to be effective in preserving the quality of the fresh produce including citrus (Ladanyia and Ladanyia, 2010). This method involves storing produce under specific environmental conditions such as reduced oxygen levels, elevated carbon dioxide concentrations, precise temperatures, and high humidity. The main advantages of CA storage include suppression of ethylene production, which delays ripening, and prevention of physiological disorders that could negatively impact the appearance, taste, nutrition, and safety of the fruit (Dilley, 2010).

Controlled atmosphere storage has been identified as an effective method for managing fungal decay in citrus fruits by utilizing high concentrations of carbon dioxide (CO_2) (Strano et al., 2022). Low CO_2 levels have been shown to lead to the accumulation of ethanol and acetaldehyde, as well as an increase in the activity of pyruvic decarboxylase and alcohol dehydrogenase in oranges, indicating metabolic changes associated with fruit storage under varying atmospheric conditions (Strano et al., 2022).

Different citrus fruits exhibit distinct requirements for optimal storage conditions within CA environments (Table 2.3). Research on 'Sanguinello Comune' blood oranges stored at 10°C under high oxygen conditions (76 kPa O_2) showed elevated levels of anthocyanins and phenolic compounds, indicating an oxidative stress response to oxygen-rich environments (Molinu et al., 2016). Conversely, 'Honey' pomelo stored under the same conditions exhibited a decline in vitamin C content and antioxidant capacity, highlighting the variability in how different citrus cultivars respond to controlled atmospheric conditions (Li et al., 2012).

Table 2.3: Controlled Atmospheric conditions required by various citrus

Varieties	**Temperature (°C)**	**Relative humidity (%)**	**O_2 (%)**	**CO_2 (%)**
Lemon	10-15	90-95	5-10	0-10
Lime	10-15	90-95	5-10	0-10
Orange	5-10	90-95	5-10	0-5

(Strano et al., 2022)

2.5 Citrus fruit disorders

Citrus fruit disorders significantly contribute to the deterioration of fruit quality, impacting both external and internal characteristics that affect edibility and appearance. According to Kassim et al. (2020), harvesting techniques play a crucial role in the development of these disorders, suggesting that improper handling during this critical stage can exacerbate quality issues. Disorders in citrus fruits are typically classified into two main categories: physiological disorders and pathological disorders.

2.5.1 Physiological disorders

Physiological disorders are primarily induced by abiotic stresses such as temperature fluctuations, humidity levels, moisture stress, and nutrient imbalances (Kalatippi & Hota, 2021). Common physiological disorders include chilling injury, stem-end rind breakdown (SERB), peel pitting, granulation, puff and crease and sunburn. These disorders may not directly affect the edibility of citrus fruits; however, they can significantly reduce nutritional value and aesthetic appeal (Vera-Guzman et al., 2017). For instance, physiological disorders can alter physicochemical properties such as titratable acidity (TA), pH, and vitamin C content, ultimately affecting the flavour profile of the fruit (Mditshwa et al., 2017).

2.5.1.1 Oleocellosis

Oleocellosis is a prevalent physiological disorder in citrus fruits, particularly characterized by the appearance of brownish to dark oily spots on the rind (Fig. 2.3). This condition typically arises during transportation and handling, where mechanical injuries—resulting from improper or rough handling—damage the oil glands within the fruit (Melgar et al., 2011). The rupture of these glands leads to the release of phytotoxic oils that infiltrate the rind, causing oleocellosis.

In addition to mechanical injury, several other factors contribute to the development of oleocellosis, including insect attacks, hail damage, bruising, and contact between damaged and undamaged fruits. The incidence of oleocellosis is most commonly observed in limes, lemons, and sweet oranges (Montero et al., 2012).

Oleocellosis impacts both visual appeal and commercial value, resulting to significant economic implications for growers and distributors due to reduced marketability. Effective management strategies are essential to minimize the occurrence of oleocellosis, including careful handling practices during harvesting and transportation to prevent mechanical damage.

Figure 2.6: Oleocellosis disorder on citrus (Montero et al., 2012)

2.5.1.2 Chilling injury

Chilling injury (CI) is a physiological disorder that occurs in tropical and subtropical fruits when they are stored at temperatures below 10-15°C for extended periods (Ashebre, 2015). This condition is characterized by the appearance of spots on the fruit surface, which develop as a consequence of severe cold treatment (Fig. 2.4). In citrus fruits, which fall under the category of tropical fruits, the susceptibility to chilling injury varies among species, cultivars, and climatic conditions (Ashabre, 2015).

Research indicates that lemons, limes, and grapefruits are more susceptible to chilling injury compared to mandarins and oranges (Golding, 2019). The development of chilling injury symptoms is closely linked to the interplay of time and temperature; symptoms typically manifest after fruits are transitioned from lower temperatures to warmer environments (Siboza et al., 2014).

The underlying mechanisms contributing to chilling injury involve alterations in the physical properties of cell membranes due to changes in the physical state of membrane lipids. Additionally, structural proteins such as tubulin and various enzymes undergo modifications that affect enzyme kinetics (Siboza et al., 2017). These biochemical changes can lead to a range of symptoms including surface pitting, discoloration, internal breakdown, and overall deterioration of fruit quality.

Figure 2.7: Chilling injury symptoms in citrus (Gerald Holmes, Strawberry Center, Cal Poly San Luis Obispo, Bugwood.org)

2.5.1.3 Peteca

Peteca is a postharvest physiological disorder predominantly affecting lemons, characterized by the collapse of oil glands in the fruit (Fig. 2.5). This collapse leads to the leakage of oil into adjacent tissues, resulting in darkened depressions on the rind (Cronje, 2016). Symptoms of peteca include rind pitting, sinking of the rind, and darkening of the oil glands. Extended periods of brushing during fruit processing have been demonstrated to heighten oleocellosis occurrence (Cronje, 2016).

Research indicates that the type of wax applied to the fruit can influence the development of peteca, with polyethylene-based waxes inducing more instances of this disorder compared to carnauba wax emulsions (Ashebre, 2015). Additionally, peteca is more likely to occur under conditions of very high humidity (Cronje, 2016). Nutritional imbalances also play a role in the manifestation of peteca; specifically, elevated nitrogen levels and moisture stress are linked to increased oxalic acid concentrations in the leaves (Cronje, 2016). Furthermore, high calcium levels coupled with low available phosphorus levels in the soil contribute to the disorder's prevalence (Ashebre, 2015).

A study conducted by Cronje (2016) on 'Eureka' lemons demonstrated that postharvest applications of Ethephon at concentrations of 200 and 400 mg/L, as well as AVG (aminoethoxy-vinylglycine) at 400 and 800 mg/L, resulted in a significant reduction in peteca incidence. These findings suggest that managing preharvest and postharvest conditions, including nutritional status and handling practices, is crucial for mitigating peteca and preserving fruit quality.

Figure 2.8: Peteca disorder on the rind of the lemon fruit (Cronje, 2016)

2.5.2 Pathological disorders

Pathological disorders are quite common in citrus fruits and are responsible for significant postharvest losses. *Penicillum digitatum*, *Penicillum italicum* and *Geotrichum candidum* which cause green mold, blue mold and sour rot, respectively, are the most economic pathogens for citrus fruit (Strano et al., 2017). Decay also makes a major contribution to the production loss as the appearance and the edibility of the fruit is affected (Özdemir, 2021).

2.5.2.1 Green mold

Green mold, primarily caused by the fungus *P. digitatum*, is a significant contributor to fruit decay, leading to considerable economic losses in agricultural sectors, particularly in arid and subtropical climates (Bhatta, 2022). The decay process is exacerbated by the presence of damaged tissues in the fruit, which release volatile compounds, organic acids, and sugars that facilitate the germination of conidia, the asexual spores of the fungus (Bhatta, 2022).

The infection of fruit by *P. digitatum* typically occurs through mechanical wounds inflicted during harvesting and sorting processes. Various environmental factors, including wind, hail, and insect activity, further contribute to these injuries (Perez et al., 2017). Once the fruit is wounded, *P. digitatum* can penetrate the tissue, leading to decay. The dispersal of conidia is primarily facilitated by wind and raindrops, which enable spores to adhere to the surface of the fruit and subsequently germinate into germ tubes (Han et al., 2017).

Fruit decay due to *P. digitatum* is particularly pronounced in arid and subtropical regions, where climatic conditions favour fungal proliferation. This phenomenon not only reduces the volume of exportable fruit but also results in significant economic repercussions for producers (Cheng et al., 2020). The cumulative effects of mechanical damage and environmental stressors underscore the vulnerability of fruit crops to fungal infections, necessitating effective management strategies to mitigate losses.

The infection of citrus fruit by *P. digitatum* begins with the penetration of germ tubes into pericarp cells, subsequently extending into mesocarp cells and invading adjacent tissues (Lin et al., 2019). This invasion is characterized by the production of white mycelia and newly formed grayish conidia on the surface of infected fruit, which are recognized as typical symptoms of citrus postharvest green mold (Han et al., 2017; Lin et al., 2019).

Initially, infected citrus fruit exhibit symptoms of waterlogging, which progresses to complete rotting. This deterioration occurs as plasmolysis affects the pericarp and mesocarp cells, leading to the coagulation and digestion of cellular inclusions and organelles (Han et al., 2017). The visible manifestation of this decay includes a soft, watery spot that develops into a more severe rotting condition.

Research has identified approximately 19 key pathogenicity factors that contribute to the virulence of *P. digitatum* on citrus fruit. These factors have been elucidated through the use of an *Agrobacterium tumefaciens*-mediated transformation system, which facilitates genetic studies of *P. digitatum* (Fig. 2.6). Understanding these factors is crucial for developing strategies to mitigate the impact of this pathogen on citrus production.

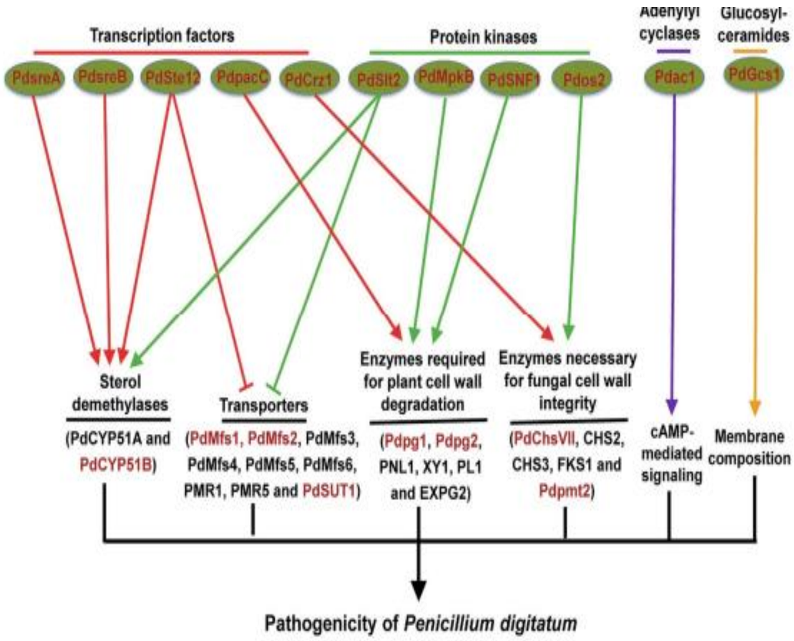

Figure 2.9: Pathogenicity of *Penicillium digitatum* (Vu et al., 2018)

2.5.2.2 Blue Mold

Penicillium italicum, the necrotrophic fungus responsible for blue mold, exhibits a rapid disease cycle and significantly impacts postharvest citrus quality (Bhatta, 2022). The pathogen, which sustains itself on dying cells, infiltrates fruit through harvest and transport-related wounds (Bhatta, 2022).

The pathogen's spores are contained within a confined white mycelium surrounded by a damp, fluffy rind (Bhatta, 2022; Papoutsis et al., 2019). The infection process involves mycelial growth into fruit tissue, culminating in blue conidia formation through sporulation (Louw & Korsten, 2015). Unlike its relative *P. digitatum* which causes green mold, blue mold demonstrates aggressive spread between fruits even in cold storage (Iqbal et al., 2017).

Blue mold infection initially manifests as water-soaked areas on the fruit surface, progressing to tissue softening and discoloration caused by pathogenic enzymes like polygalacturonase and glucosidase (Papoutsis et al., 2019). Advancing infection weakens rind tissue, increasing susceptibility to physical damage (Louw and Korsten, 2015). Disease development is modulated by fruit ripeness, temperature conditions (optimally 20–25°C), and wound-based spore concentration (Papoutsis et al., 2019).

According to the study conducted by Louw and Korsten (2015), it was noted that *P. italicum* caused lesions of about 33.9 ± 11 mm on ‘Eureka’ lemons stored at ambient temperatures with 4.8mm/day daily growth rate. However, under cold storage, it caused smaller lesions of about

19.9 ± 11.0 nm with 1.4 mm/day daily growth rate. The infected fruits under cold storage started showing signs after 12 days while under ambient conditions showed signs after 2-3 days.

P. italicum exhibits a unique ability to proliferate in cold storage conditions, where it can outcompete other pathogens like *P. digitatum* (Iqbal et al., 2012). The spread of this pathogen occurs through airborne spores that can contaminate healthy fruits through direct contact with wounded areas before or after harvest (Papoutsis et al., 2019).

Recent studies have highlighted the development of fungal resistance in *P. italicum*, particularly concerning its response to sterol demethylase inhibitors (DMIs), a class of fungicides commonly used in the agricultural industry. Zhang et al. (2020) conducted a comparative analysis of two strains of *P. italicum*: one strain exhibited high resistance to DMIs, while the other was highly sensitive (Zhang et al., 2019). This differential response is attributed to specific genetic factors associated with the resistant strain.

The resistance observed in the highly resistant strain is linked to several ergosterol biosynthesis-related genes, including ERG2, ERG11 (CYP51 isoform), and ERG6. These genes encode enzymes such as sterol isomerase, sterol 14-α-demethylase, and sterol methyltransferase, which are crucial for the synthesis of ergosterol, a key component of fungal cell membranes (Zhang et al., 2020). The upregulation of these genes suggests that alterations in ergosterol biosynthesis may confer resistance by enabling the fungus to mitigate the inhibitory effects of DMIs.

In addition to ergosterol biosynthesis genes, the study identified various other proteins that may play a role in resistance mechanisms. These include ATP-binding cassette (ABC) transporter family proteins, multi-drug and toxic compound extrusion (MATE) family proteins, and major facilitator superfamily (MFS) proteins, all of which were found to be upregulated in the resistant strain (Zhang et al., 2020). The presence of these transporters suggests an enhanced ability of the resistant strain to efflux toxic compounds, thereby reducing the efficacy of fungicides.

2.5.2.3 Sour Rot

Sour rot, primarily caused by the fungus *Geotrichum candidum*, leads to rapid deterioration in the quality of citrus fruits. This disease is particularly exacerbated during periods of heavy rainfall, as healthy fruits can become damaged through contact with infected ones (Saito & Xiao, 2017). The symptoms pertaining the development of sour rot include soft rot, non-discoloured lesions with dark-brown areas and purple margins, combined with a distinct sour odour (Francois et al., 2022). The disease is more apparent in instances of long storage and affects all varieties of citrus with mandarins being more prone to the disease as compared to other citrus varieties (Feraz et al., 2016; Nazerian and Alian, 2013).

Fungi are recognized for their capacity to synthesize metabolites that find applications across various sectors, including pharmaceuticals. In a study by Qi et al. (2018), a total of 53 metabolites were identified, with 9 exhibiting upregulation and 43 downregulation. Pyruvate is crucial in metabolic pathways, facilitating the connection between carbohydrates, amino acids, and fatty acids (Francois et al., 2022). The aromatic amino acids synthesized by *G. candidum* have been identified as precursors to antimicrobial compounds such as phenyllactic acid (PLA), which demonstrates efficacy against *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* (Naz et al., 2013; Kawtharani et al., 2020). PLA has been shown to induce structural changes in microorganisms, including *L. monocytogenes* and *Fusarium* species (Kawtharani et al., 2020). Additionally, indoleacetic acid (IAA), a metabolite produced by endophytic fungi, plays a significant role in enhancing plant resilience to abiotic stressors such as salinity and drought (Francois et al., 2022). Furthermore, IAA is reported to promote plant growth, flowering, and the development of roots and axillary buds (Lubna et al., 2018).

Research on *G. candidum* has predominantly concentrated on its primary metabolites, while the exploration of its secondary metabolites remains insufficiently addressed (Qi et al., 2018; Ząbek et al., 2017).

2.6 Postharvest Treatments

Adoption of postharvest management strategies has grown substantially, driven by their effectiveness in extending storage durability of degradable products, particularly citrus. Postharvest treatments can be classified into several categories; however, this review will specifically concentrate on chemical treatments, physical treatments, edible coatings, and gaseous treatments, all of which have demonstrated efficacy in extending the shelf-life of citrus products (Palou, 2013; Pistijono et al., 2018).

2.6.1 Chemical treatments

2.6.1.1 1-Methylcyclopropene (1-MCP)

1-Methylcyclopropene (1-MCP), commercially recognized as SmartFresh®, is a synthetic compound utilized as an ethylene inhibitor to modulate the ripening process in fruits (Bambalele et al., 2021). By binding preferentially to ethylene receptors, 1-MCP disrupts the action of both endogenous and exogenous ethylene, subsequently affecting the activity of cell wall-lysing enzymes and delaying fruit softening (Wei et al., 2020). The application of 1-MCP has been extensively studied, highlighting its potential benefits in post-harvest management.

Research by Li et al. (2012) on 'Shatangju' mandarin treated with 1-MCP (5 $\mu L\ L^{-1}$) for 10 days showed no substantial effect on respiration patterns, contrasting with findings by Cajuste et al. (2011) who observed significant respiratory changes in grapefruits and oranges. While Li et al. (2012) found no immediate changes in fruit characteristics, they observed temporary increases in ascorbic acid and titratable acidity by Day 10, though these differences normalized by the storage period's end. The treatment resulted in reduced total soluble solids compared to untreated fruit.

1-Methylcyclopropene has demonstrated efficacy in mitigating weight loss in citrus fruits. Baswal et al. (2020b) observed that ‘Kinnow’ mandarin treated with 1.5 µL/L 1-MCP exhibited a weight loss of only 3.2%, in contrast to an 8.4% loss in the control group after storage for 30 to 75 days. This weight loss is primarily attributed to transpiration and respiration processes occurring post-harvest (Hung et al., 2011). Additionally, the juice content of the treated fruits was significantly higher, measuring at 41.6% compared to 37.8% in the control, which is favourable in market preferences due to the higher juice yield resulting from reduced moisture loss during storage (Baswal et al., 2020b). Furthermore, firmness in citrus fruits serves as an indicator of pectin activity, with its degradation being associated with the ripening process (Frempong et al., 2022). Baswal et al. (2020b) reported that treatment with 1-MCP not only enhanced firmness retention but also correlated with elevated levels of pectic substances and diminished activity of pectin methylesterase in the treated fruits.

2.6.1.2 Salicylic Acid (SA)

Salicylic acid (SA) is a crucial phytohormone in plants, recognized for its significant roles in modulating responses to biotic stresses, facilitating plant development, and enhancing defense mechanisms (Deng et al., 2022). Research has demonstrated that SA can activate various genetic pathways in citrus fruits, contributing to their disease resistance. Notably, genes such as WRKY3, WRKY72, WRKY38/62, and WRKY46 from *Arabidopsis thaliana*, along with WRKY30, WRKY45, and WRKY23 from *Oryza sativa*, have been implicated in this process (Deng et al., 2020).

In a study by Deng et al. (2020), the impact of SA on *P. digitatum* was evaluated using citrus fruit (*Citrus sinensis* L. Osbeck cv. Jincheng) treated with aqueous solutions of SA at concentrations of 1, 2, or 4 mmol/L. The treated fruits were individually packaged in plastic bags and stored at 25 °C with 90–95% relative humidity. The findings revealed that the incidence of disease in fruits treated with 2 mM SA was reduced to 34%, compared to 51% in the control group when assessed 121 hours post-inoculation. To elucidate the underlying mechanisms of SA-induced resistance in fruits against fungal pathogens, transcriptomic analyses were conducted. These analyses indicated that genes upregulated by SA were significantly enriched in functions related to disease resistance.

Similar to 1-Methylcyclopropene (1-MCP), salicylic acid (SA) can be employed alongside other treatments to enhance fruit quality. Siboza et al. (2014) studied how SA and methyl jasmonate (MJ) impacted 'Eureka' lemons. The fruits underwent treatment with either 2 mM SA, 10 μM MJ, or both combined. Post-treatment, the lemons were waxed and kept at −0.5, 2, or 4.5°C for up to 28 days, then 7 more days at 23°C. The findings indicated that the combined application of MJ and SA significantly mitigated chilling injury in the lemons. Furthermore, the treated fruits exhibited elevated levels of phenolic compounds, irrespective of the treatment concentration. Variations in phenolic content were attributed to the specific treatments applied, storage conditions, and preharvest practices, all of which are critical in influencing the antioxidant capacity of the fruit (Siboza et al., 2014). Additionally, untreated fruits demonstrated lower levels of and phenylalanine ammonia-lyase and peroxidase enzymes compared to their treated counterparts, which showed significantly increased enzyme activity.

2.6.1.3 Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is recognized for its efficacy in mitigating various postharvest disorders, particularly those associated with fungal decay (Cerioni et al., 2013; Waghmare & Annapure, 2017). Its application in fresh-cut fruits is attributed to its capacity to preserve and enhance the quality of the produce by preventing decay. However, challenges arise from the instability of pure hydrogen peroxide, which limits its standalone use. Consequently, it is often combined with other agents, such as acetic acid and silver-ions, to create a more effective and stable disinfectant formulation (Sisquella et al., 2013; Cerioni et al., 2013).

The study conducted by Meng et al. (2019) demonstrated that hydrogen peroxide (H_2O_2) significantly affected the quality of orange fruits examined in their study. Oranges treated with H_2O_2 exhibited fewer lesions compared to those treated with alternative chemical agents. Specifically, concentrations of H_2O_2 exceeding 0.5% completely inhibited the in vitro growth of *P. digitatum* and *P. italicum*, while concentrations above 0.25% effectively eliminated all bacteria, mold, and yeast present on the fruit's surface. However, it was noted that during prolonged storage, a higher concentration of H_2O_2 (2%) was required for decay control, although this was still less effective than imazalil. Consequently, H_2O_2 appears to be more efficacious in preventing decay during short-term storage periods.

2.6.2 Non-chemical treatments

2.6.2.1 Hot water treatments (HWT)

The application of postharvest thermal treatments as an alternative to fungicide use has demonstrated promising efficacy in controlling fungal development in various fruits, including citrus species (Sui et al., 2016). Numerous studies have investigated the effectiveness of hot water treatments on different fruits and vegetables. For example, Revynthi et al. (2020) conducted experiments where lemons were immersed in hot water at temperatures of 21, 48, 53, or 63°C for durations of 5, 10, and 15 minutes. The results indicated that immersions at 53°C and 63°C across all time intervals successfully eliminated over 99% of adult mites. However, quality assessments revealed that fruits subjected to higher temperatures (63°C and 53°C) experienced significant quality deterioration compared to those treated at lower temperatures.

Maintaining optimal temperatures is crucial, as fruits possess specific temperature thresholds beyond which their structural integrity may be compromised. Elevated temperatures can lead to cell wall degradation, resulting in a decline in fruit quality. Research indicates that hot air treatment can enhance oxygen scavenging and facilitate the solubilization of cell wall polysaccharides, thereby improving the cold tolerance of loquat fruits (Shao & Kang, 2014).The application of hot water treatment (HWT) presents several advantages, notably its ability to mitigate surface infections that penetrate the fruit skin without leaving harmful chemical residues.

As a chemical-free technique, “hot water treatment (HWT)” is acknowledged for prolonging fresh produce shelf-life. Fungal and microbial infestations significantly diminish the storage longevity of sweet oranges. HWT is a postharvest technique that can enhance the shelf-life of fruits by reducing postharvest respiration and other metabolic processes (Ullah et al., 2018). However, research by Ullah et al. (2018) on ‘Sherkhana-I’ sweet oranges revealed that immersion in hot water at a temperature of 45 ± 2 °C resulted in a reduction of fruit firmness, attributed to water absorption and subsequent weight loss. The temperature affects cellular turgor, and the observed decrease in firmness may indicate pectin degradation, which is crucial for maintaining structural integrity (Baswal et al., 2020b). Furthermore, a decrease in vitamin C, total soluble solids (TSS), and titratable acidity (TA) was detected, which was associated with weight loss and respiration rates throughout storage (Ullah et al., 2018).

Kaewsuksaeng et al. (2015) conducted a study on 'Thai' lime subjected to hot water treatment at 50°C for durations of 3 and 5 minutes, followed by storage at 25°C in darkness. The findings indicated that a 5-minute hot water treatment significantly delayed the reduction of hue angle values and chlorophyll a content. In the control group, titratable acidity (TA) decreased after 5 days of storage, whereas in the treated group, TA exhibited a continuous increase every 5 days over a period of 35 days. Additionally, total soluble solids in the control group rose markedly on Day 10 before declining during storage. These results provide substantial evidence supporting the efficacy of hot water treatment as a non-chemical method currently employed in the fresh produce sector to uphold quality and prolong shelf-life.

2.6.2.2 Hot air treatment

Hot air treatment (HAT) significantly affects both postharvest pathological and physiological disorders in citrus fruits. The process, defined as the exposure of air to temperatures ranging from 37 to 45°C for a duration of 2 to 3 days, is referred to as curing (Strano et al., 2022). There is a need for further investigation into the impact of HAT on the postharvest quality of citrus fruits, as existing studies present varying conclusions regarding its efficacy. Specifically, HAT has been shown to enhance the expression of genes such as CitAco2/3, CitIDH2/3, and CitGAD4, which are involved in the degradation of citric acid in 'Ponkan' mandarin fruit (Gao et al., 2018). The degradation pathways for citric acid include the acetyl coenzyme A pathway (ACL), glutamine pathway (GS), and gamma-aminobutyric acid (GABA) pathway (Gao et al., 2018).

Hot air treatment (HAT) shares critical factors of time and temperature with hot water treatment (HWT). Research has demonstrated that HAT can effectively enhance disease resistance in fruits. For example, a study by Liu et al. (2022) on ‘Baizhong' papaya revealed that applying forced hot air at 47.2°C for intervals of 30, 60, and 90 minutes, followed by storage at 20°C with 80% relative humidity (RH), significantly improved the fruit's resistance to fungal pathogens such as *Colletotrichum gloeosporioides*.

The application of hot air treatment (HAT) not only reduces the incidence of diseases but also modifies physiological characteristics such as firmness and acidity, which are essential for consumer acceptance. It can be inferred that HAT facilitates the redistribution of natural epicuticular wax on the fruit surface, thereby creating a gas barrier that may mitigate the oxidation of ascorbic acid (Mditshwa et al., 2017). Additionally, HAT is thought to lower the respiration rate of fruits, consequently delaying their deterioration (Gao et al., 2018).

2.6.2.3 Irradiation

Irradiation is a non-thermal safe technology approved by the World Health Organization, Food and Agriculture Organization for postharvest treatment of fresh fruit and vegetables (Deepshikha et al., 2017). Irradiation can be applied by non-ionizing radiations such as ultraviolet light (Komolprasert, Packaging food for radiation processing, 2016). The effect of irradiation on citrus fruit has previously been evaluated even though it has received little attention from postharvest technologists and researchers. Abdullar et al. (2018) evaluated how different radiation dosages affected 'Kinnow' mandarin quality. They discovered that a 1.5 kGy dose reduced mold incidence more effectively than doses of 0.0, 0.25, 0.50, 0.75, 1.0, and 2.0 kGy. Furthermore, gamma-ray irradiation below 1 kGy has demonstrated potential as a postharvest phytosanitary measure for eradicating quarantine pests in Korean citrus fruits (Jo et al., 2018).

There is still however little literature on the influence of irradiation treatment on citrus fruits. For instance, the specific mechanisms by which irradiation affects the physiological and biochemical processes in citrus fruits remain poorly understood. Research has shown that irradiation can induce resistance to pathogens and alter antioxidant properties, yet the underlying biological pathways and how these changes contribute to fruit quality and shelf-life need further studying (Zhao et al. 2020; Strano et al. 2022). In addition to that, while studies by Bermejo at al. (2011) and Borghi & Pavanelli (2023) indicate that gamma irradiation can affect various quality attributes of citrus fruits, such as fruit size, acidity, and nutritional composition, there is however limited understanding of how these changes impact the overall nutritional quality and health benefits of the fruits. For instance, some irradiated clones of citrus show variations in ascorbic acid and flavonoid content, but comprehensive studies comparing these changes across different cultivars are lacking.

2.6.2.4 UV-C irradiation

Ultraviolet (UV) irradiation, a common method for reducing rot, has also proven effective in postponing fruit senescence and promoting the synthesis of beneficial compounds (Gündüz & Pazir, 2013). In cold-stored citrus fruits, resistance induction has been linked to the accumulation of phytoalexins like scopoletin and scoparone (Strano et al., 2022). Phonyiam et al. (2021) showed that treating 'Satsuma' mandarins with UV-C radiation at 10 kJ m^{-2} successfully suppressed *Penicillium digitatum* growth. This treatment maintained membrane integrity by decreasing lipid peroxidation and stimulating jasmonic acid accumulation, which consequently triggered the production of bioactive compounds and improved the antioxidant potential, as determined by DPPH radical scavenging capacity.

Yamaga et al. (2016) demonstrated that low doses of UV-B radiation (15 kJ m^{-2}) effectively inhibited the growth of *P. italicum* in vitro, resulting in a reduction of spore germination by over 99%. However, due to the limited penetration abilities of irradiation techniques on fruit surfaces, it is essential to integrate these methods with alternative strategies. The combination of integrated applications, including Generally Recognized As Safe (GRAS) compounds, heat treatments, cold storage, and the use of antagonistic microorganisms, has proven to be an effective approach. These synergistic effects enhance overall efficacy and yield better results in postharvest management (Strano et al., 2022).

2.6.2.5 Gamma-ray irradiation

Commercial applications of phytosanitary irradiation for the elimination of quarantine pests in agricultural commodities are on the rise, particularly in citrus fruits, driven by high market demand due to their widespread consumption (Jo et al., 2018). The practical applications of food irradiation can be categorized based on radiation doses into three distinct groups: low dose applications (up to 1 kGy), medium dose applications (1 kGy to 10 kGy), and high dose applications (above 10 kGy). Low doses are primarily employed for sprout inhibition in bulbs and tubers, with doses of 0.25-1.0 kGy used to delay fruit ripening and 0.1-1.0 kGy for insect disinfestation and the destruction of foodborne parasites (Haider & Naveed, 2023).

According to Jo et al. (2018), gamma irradiation at doses up to 1 kGy had marginal impact on weight loss rates, firmness, total soluble solids, fructose, glucose, vitamin C, hesperidin, citric acid, malic acid, succinic acid, and sensory attributes of ‘Jinjihyang’ oranges and ‘Chunggyun’ mandarins. However, it induced significant changes ($p < .05$) in flavonoid content, specifically narirutin (ranging from 94.80 to 109.64 mg/100 g), sucrose (7693–7732 mg/100 mL), and titratable acidity (0.65–0.72%) in ‘Jinjihyang’ oranges, while no significant effects were observed in ‘Chunggyun’ mandarins. The two citrus varieties exhibited different responses to irradiation, with storage at 4 ± 1 °C having a more pronounced effect on quality attributes than the irradiation itself. Although gamma irradiation at 1 kGy improved the shelf stability of citrus fruits, the optimal absorbed dose varied between the fruit types. These findings suggest that gamma irradiation up to 1 kGy can be effectively utilized for phytosanitary purposes without adversely affecting the major physicochemical and sensory attributes of these Korean citrus fruits during long-term storage.

2.6.3 Edible coatings

Edible coatings (EC), often referred to as "environmentally friendly" coatings, are thin layers of consumable substances applied to the surfaces of produce, ensuring safety at concentrations effective for preserving quality (Dhall, 2013). The use of edible coatings derived from plant extracts (ECPE) is essential for maintaining horticultural produce quality (Murmu & Mishra, 2018; Tesfay & Magwaza, 2017). The application of animal-derived extracts faces challenges due to consumer allergies, religious dietary restrictions, and the potential for these substances to form impermeable layers due to their waxy properties (Shiekh et al., 2013). In contrast, ECPE typically consists of extracts from plants recognized as safe for human consumption, such as fruits, herbs, and spices that possess high antioxidant and antimicrobial properties (Florez-López et al., 2016). Consequently, these coatings may offer additional medicinal benefits upon consumption.

2.6.3.1 Chitosan

Chitosan, a natural polymer derived from the deacetylation of beta 1,4-D-glucosamine, exhibits biodegradable and nontoxic properties along with notable antimicrobial characteristics (Gurjar et al., 2018). This makes chitosan an effective agent for inhibiting postharvest diseases and enhancing the quality of fresh fruits (Bambalele et al., 2018). For instance, research by Arnon et al. (2014) demonstrated that chitosan significantly improved the overall quality of citrus fruits.

Arnon et al. (2014) found that chitosan coatings exhibit water resistance, enabling them to endure cold storage and shelf-life conditions effectively. This characteristic prevents excessive carbon dioxide buildup and avoids creating anaerobic conditions within the fruit's internal atmosphere. Notably, the application of chitosan significantly enhanced the glossiness and overall appearance of the treated fruit. The coating forms a homogeneous, translucent layer that adequately covers the fruit surface. Additionally, being derived from polysaccharide materials, chitosan is relatively inexpensive and economical to use.

The findings observed by Arnon et al. (2014) align with the assertions made by Dhall (2013) regarding the efficacy of chitosan as a coating for postharvest handling of citrus fruits. Chitosan coatings are homogeneous, stable, relatively inexpensive, and easy to prepare, making them a practical choice for enhancing fruit quality. Importantly, the use of chitosan does not necessitate organic solvents such as ammonia, rendering it safe and biodegradable.

2.6.3.2 Carboxymethyl cellulose (CMC)

Among the various edible coatings utilized in the food industry, moringa, chitosan (CH), and carboxymethyl cellulose (CMC) are among the most prevalent. Research by Adetunji et al. (2013) (Adetunji, et al., 2013) indicated that the incorporation of moringa with CMC effectively extended the shelf- life and maintained the quality of oranges. Similarly, Tesfay and Magwaza (2017) evaluated the effectiveness of chitosan and CMC combined with moringa on the postharvest quality of avocados, finding that these coatings improved fruit quality and extended shelf-life after 21 days of cold storage at 5.5°C. Notably, their study highlighted that CMC containing moringa extract was particularly effective in suppressing postharvest diseases and preserving avocado quality.

Extended shelf-life has also been reported in guavas treated with a chitosan-cassava starch coating prior to storage (de Aquino et al., 2015). Furthermore, chitosan and CMC coatings have been evaluated across various citrus fruits, including ‘Navel’ oranges, ‘Star Ruby’ grapefruit, and mandarins. These studies consistently demonstrated that such coatings enhance fruit firmness while sensory evaluations indicated that fruit flavour remained unaffected (Arnon et al., 2015).

The effectiveness of these edible coatings can be attributed to their ability to create a barrier that reduces moisture loss and gas exchange, thereby maintaining fruit firmness and quality during storage. The incorporation of natural extracts like moringa not only provides antimicrobial properties but also enhances the nutritional profile of the coatings, making them a sustainable choice for postharvest management in the fruit industry.

2.6.4 Gaseous treatments

2.6.4.1 Ethylene

Citrus fruits are classified as non-climacteric, meaning that their postharvest development does not involve increased rates of respiration or ethylene production. However, exposure to exogenous ethylene can effectively stimulate certain ripening processes in citrus fruits. For example, it can limit the production of chlorophyll pigments, which contribute to the green coloration of the fruit, while promoting the accumulation of carotenoids responsible for the orange and yellow hues (Morales et al., 2023).

Research indicates that exposure to ethylene enhances chlorophyllase activity and increases the expression of genes involved in chlorophyll breakdown (Yin et al., 2016). Additionally, ethylene stimulates the transcription of carotenoid biosynthetic genes that facilitate the transformation of chloroplasts into chromoplasts, leading to colour changes in the fruit (Morales et al., 2023). Ethylene also represses the expression of genes associated with Mg-chelatase and other photosynthesis-related genes, thereby down-regulating chlorophyll biosynthesis (Morales et al., 2023).

Ethylene is mostly applied in packhouses where the handling and packaging of the fruits take place. According to Miller et al. (2018), low concentrations of ethylene (1-5 ppm) cause colour change on the peel of the citrus fruits. Notably, Chaudhary et al. (2017) highlighted that high ethylene concentrations have no effect on colour change but instead cause multiple physiological disorders which accelerate senescence. In their study on ‘Rio Red’ grapefruit, Chaudharry et al. (2015) noticed that fruit exposure to ethylene had no effect on the physicochemical properties (firmness, TSS and acidity). However, Morales et al. (2020) reported that treating ‘Owari’ mandarin with 2 $\mu L\ L^{-1}$ of ethylene in a controlled chamber at 21 °C and 95% relative humidity (RH) for periods ranging from 2 to 8 days, depending on the initial colour of the fruit, resulted in higher total soluble solids (TSS) and an increased rate of softening. This indicates that the effects of ethylene on citrus fruits are influenced by several factors, including the specific cultivar, temperature, and duration of exposure (Morales et al., 2023; Sdiri et al., 2013).

2.4.6.2 Modified Atmosphere Packaging

Modified Atmosphere Packaging (MAP) utilizes various types of packaging materials, such as Xtend®, polyethylene, and polyvinyl chloride films, to mitigate the development of postharvest physiological and pathological disorders in fruits (Strano et al., 2022). MAP functions by replacing the atmospheric air surrounding the fruit within a sealed package with specific levels of oxygen (O_2) and carbon dioxide (CO_2) that are conducive to slowing down the onset of these disorders (Strano et al., 2022). The reduction of O_2 and CO_2 levels decreases respiration rates and fungal development, while maintaining high relative humidity helps minimize water loss, thereby reducing overall weight loss (Chaudhary et al., 2015).

MAP has proven particularly beneficial in citrus fruit production. For example, research conducted by Jawandha et al. (2014) found that ‘Baramasi’ lemons packed in low-density polyethylene (LDPE) bags preserved superior fruit quality, characterized by high sensory quality, juice content, acidity, and minimal spoilage or physiological weight loss during 50 days of ambient storage.

Baswal et al. (2020b) evaluated the impact of various film sizes of low-density polyethylene (LDPE) on the quality of 'Kinnow' mandarins stored at 5–7 °C and 90–95% relative humidity (RH) for 75 days. The study revealed variability among different modified atmosphere packaging (MAP) treatments regarding weight loss, spoilage, juice content, firmness, pectin content, activities of fruit softening enzymes, total soluble solids (TSS), and titratable acidity (TA). The observed maintenance of quality was attributed to the storage conditions and the presence of holes in the films, which helped delay water loss and spoilage by reducing respiration rates. Among the treatments, PP-film (25 μ) with pinholes was found to be the most effective in preserving various quality parameters, including soluble solid concentration (SSC), TA, ascorbic acid levels, and sensory attributes.

The improved quality under MAP was closely linked to a reduction in the activities of fruit softening enzymes such as pectin methylesterase (PME) and cellulase. Additionally, higher concentrations of CO_2 within the packaging have an antagonistic effect on ethylene biosynthesis, which indirectly delays ripening and extends shelf-life. This indicates that MAP can effectively enhance the postharvest quality of citrus fruits by creating an optimal storage environment that minimizes deterioration while preserving desirable sensory characteristics.

2.4.6.3 Low Oxygen Storage

Low oxygen storage (LOS) represents an innovative technology that facilitates the preservation of fresh horticultural products by maintaining extremely low levels of oxygen (Wright et al., 2015). Research indicates that LOS significantly reduces the incidence of decay, thereby enhancing the quality of fresh fruits (Archer et al., 2021). Specifically, oxygen concentrations below 1% have been shown to effectively inhibit mold growth, which negatively impacts fruit quality. However, it is important to note that these low oxygen levels do not eradicate fungal spores; rather, they allow for continued fungal growth once normal oxygen levels are restored (Burg, 2014). In experiments conducted by Archer et al. (2021) on oranges, it was determined that the combination of low oxygen and low-pressure treatments resulted in a reduction of weight loss in the fruits.

Although low oxygen (LO) treatment has beneficial effects on fruit quality, Archer et al. (2021) observed that elevated ethanol concentrations within the fruit may adversely affect sensory attributes. Increased levels of ethanol are associated with the development of off-flavours, rendering the fruit less appealing to consumers. Furthermore, a 1% oxygen concentration was found inadequate for the complete eradication of *P. italicum* and *P. digitatum*; under optimal conditions, these molds can proliferate, leading to spoilage. Archer et al. (2021) concluded that the duration of storage significantly influences the efficacy of oxygen treatment in preserving quality and extending shelf-life.

Citrus is a growing and important industry and LOS is an emerging technology with great potential. The effect of LOS on citrus fruit is yet to be studied. Moreover, the combinational use of LOS with other treatments such as edible coatings must be explored. It is therefore critical the potential of LOS technologies for citrus use is investigated in the next five years as tremendous results have already been reported for other fruit types.

2.4.6.4 Gaseous Ozone (O_3)

Ozone (O_3) is a triatomic molecule composed of three oxygen atoms (Shah et al., 2019). It is considered safe for use on fruits as it does not leave chemical residues on their surfaces and poses no harm to human health (Patil & Bourke, 2012). Ozone is a colorless gas that can be detected at concentrations ranging from 0.01 to 0.05 ppm (Patil & Bourke, 2012). Classified as an unstable compound, ozone decays rapidly and has a relatively short lifespan (Bambalele et al., 2021). Research indicates that ozone is effective in eliminating fungal pathogens while also preserving juice content, total soluble solids (TSS), titratable acidity (TA), and firmness in fruits (Gartia-Martin et al., 2018; Shezi et al., 2020).

Ozone (O_3) has consistently been recognized for its effectiveness in post-harvest treatment, as it does not adversely affect the quality of fruit. In a study conducted by Gartia-Martin et al. (2018), the impact of ozone at a concentration of 1.6 mg kg^{-1} administered for 12 hours per day was evaluated on 'Fortune' and 'Ortanique' oranges, as well as four other cultivars of *Citrus sinensis* (Navelate, Lanelate, Salustiana, and Valencia). The results demonstrated that ozone effectively inhibited the growth of *Penicillium digitatum* and *Penicillium italicum* across all tested varieties, highlighting its potential to suppress the development of blue and green molds. The antimicrobial properties of ozone are attributed to its ability to inhibit fungal growth, as noted by Bambalele et al. (2021).

Previous research has demonstrated that ozone (O_3) treatment enhances the antioxidant capacity of fresh fruits, with its oxidative properties potentially influencing the activities of defense-related enzymes (Zhu et al., 2019; Zhao et al., 2013; Lin et al., 2019). This enhancement may be linked to increased activity of enzymes such as phenylalanine ammonia-lyase, peroxidase, and superoxide dismutase, or a reduction in polyphenol oxidase activity (Lin et al., 2019; Chen et al., 2019; Luo et al., 2019; Petriccione et al., 2018). These enzymes can degrade the polymers present in fungal cell walls, thereby inhibiting fungal growth and reducing decay incidence in fruits (Lin et al., 2019; Luo et al., 2019). The observed increase in antioxidant capacity is attributed to the activation of various intracellular enzymes that regulate the endogenous antioxidant defense mechanisms in harvested fruits (Zhu et al., 2019).

Ozone (O_3) is recognized for its ability to reduce respiration rates and enhance flavonoid content in fresh produce (Zhu et al., 2019). For instance, Zhu et al. (2019) investigated the effects of ozone on 'Satsuma' mandarin and found that ozone treatment significantly lowered respiration rates. This effect is attributed to ozone's capacity to generate reactive oxygen species (ROS), which lead to stomatal closure and consequently reduce respiration rates. Additionally, ozone plays a role in maintaining the color of fresh produce, as it reacts with carotenoids, resulting in a bleaching effect on orange peel (Bermudez-Aguirre & Barbosa-Canovas, 2013).

While the influence of O_3 on other citrus varieties has been evaluated, there is limited literature on the influence of O_3 on 'Eureka' lemons. Moreover, the conducive concentration and treatment time to preserve the quality of 'Eureka' lemons is currently not known.

2.7. Conclusion

Citrus fruits, similar to many other fresh horticultural products, exhibit a relatively short shelf-life, necessitating the implementation of strategies to mitigate quality deterioration. Various treatments have been explored, with many demonstrating effectiveness in extending the postharvest lifespan of citrus fruits. Nonetheless, substantial losses occur due to inadequate storage conditions and the limited efficacy of existing postharvest technologies. Ozone has emerged as a promising agent for postharvest applications; however, there is currently a lack of evidence regarding its effectiveness when combined with edible coatings for controlling postharvest diseases and preserving citrus fruit quality. Research indicates that ozone can maintain the quality of citrus fruits without adverse effects on human health. Nevertheless, the specific effects of ozone on the quality of 'Eureka' lemons remain unexamined. Therefore, further investigation into the impact of non-chemical postharvest treatments, such as low-oxygen storage (LOS) and ozone, on the postharvest quality of citrus fruits is imperative.

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Chapter 3

The effect of short-term gaseous ozone treatment on physical attributes of 'Eureka' lemons during cold storage

Abstract

The current study investigated the effect of gaseous ozone on decay incidence and physical quality attributes such as mass loss, colour and firmness of 'Eureka' lemons. 'Eureka' lemons were exposed to 0.3 ppm of gaseous ozone for 24 and 48 hours while the control fruit was left untreated. Fruits were thereafter stored at 0°C, 95% RH for 28 days and thereafter exposed at ambient temperature for 7 days. The parameters were assessed at 14-day intervals. Fruit colour was measured using chromometer, while handheld penetrometer was used for assessing firmness. The findings showed that the ozone treatments significantly ($p < 0.001$) affected the decay and firmness of the 'Eureka' lemon. O_3(48h) treatment exhibited a higher decay development of 4% as compared to the O_3(24h) and the control which were 3.2% and 0.91%, respectively. The impact of the treatments varied among the studied parameters, however, O_3(48h) positively influenced colour and firmness as there were minimal losses as compared to the other treatments. There is however a need to conduct investigations on the impact of ozone on specific pathogens causing fruit decay in lemons and how ozone affects the cell wall degrading enzymes. The possibility of combinational use of gaseous ozone and edible coatings should also be investigated.

Keywords: Ozone, storage, postharvest, physical attributes, quality, shelf-life.

3.1 Introduction

The lemon (*Citrus limon*) is a important fruit due to its extensive applications across various sectors, including the food, medical, pharmacological, and cosmetics industries (Lorente et al., 2014; Arbona et al., 2015; Sánchez-Bravo et al., 2022). This significance is attributed to its abundant bioactive compounds, which encompass phenolic compounds, carotenoids, vitamins A, C, and B, minerals, citric acid, and essential oils (Mamede et al., 2020; Turner & Burri, 2013).

Citrus by-products, particularly peels, represent a promising economic source of bioactive ingredients with notable nutritional properties (Hayam & Ahmed, 2018). These compounds play critical roles in fruit senescence and decay resistance while also providing health benefits to humans (Serna-Escolano et al., 2019). Notably, the concentrations of total phenols and carotenoids in lemon peels surpass those found in the flesh of the fruit (Ramful et al., 2011; Sharma et al., 2017). Beyond biochemical characteristics, physical quality attributes such as color, freshness, and firmness are essential for consumer acceptance (Serna-Escolano et al., 2023). Numerous studies have indicated that various factors, including storage conditions and treatment methods, significantly influence the physicochemical properties of citrus fruits (Ahmed & Azmat, 2019; Aguilar-Hernández et al., 2020; Martínez-Nicolas et al., 2022; Mukhim et al., 2015).

In recent years, fresh produce growers have shown increasing interest in utilizing ozone (O_3) due to its self-decomposing nature and potent oxidative properties (Sarron et al., 2021; Sivaranjani et al., 2021; Pandiselvam et al., 2019). Ozone functions as a powerful disinfectant owing to its triatomic oxygen structure. The three oxygen atoms are arranged at an obtuse angle, with an included angle between two attached oxygen atoms measuring approximately 116∘8′116∘8′ and a bond length of 1.278 Å (Sarron et al., 2021; Sivaranjani et al., 2021; Pandiselvam et al., 2019).

Ozone demonstrates significant disinfectant capabilities and can effectively inactivate pathogenic bacteria such as *Escherichia coli*, *Salmonella typhimurium*, and *Listeria monocytogenes*, as well as viruses and protozoa (Song et al., 2015; Brié et al., 2018). Importantly, unlike many postharvest treatments, ozone does not leave harmful residues on the treated produce. The study by Xue et al. (2023) indicates that ozone can react with other substances such as pesticide residues in grains (Sivaranjani et al., 2021) and pollutants in wastewater (Margot et al., 2013), owing to its high oxidizing capacity. Despite extensive studies on ozone's effects on various fresh fruits and vegetables, there remains limited information regarding its impact on decay incidence and physical quality attributes specifically in citrus fruits like lemons. Therefore, this study aims to investigate the effects of gaseous ozone on key physical quality properties of ‘Eureka’ lemons.

3.2 Materials and method

3.2.1 Fruit material

Eureka’ lemons were procured from Carisbrooke Citrus Valley Farm, Ixopo, KwaZulu-Natal (30.24172° S, 30.01315° E). Upon arrival at the Postharvest Laboratory of the Department of Horticultural Science, fruit were graded for uniformity according to colour and size, and thereafter stored in carton boxes. The experiment was arranged in a complete randomised design, in which each treatment had five replicates with each replicate containing 22 fruits.

3.2.2 Ozone treatment and cold storage

The fruit were treated with 0.3 ppm gaseous ozone equipped with an ozone analyzer (Corona discharge ozone generator, Ozone Purification Technology, Johannesburg, South Africa). Fruits were assigned to respective gaseous ozone treatments where time was the treatment factor: T1: Control (untreated lemons); T2: 0.3 ppm (24h); T3: 0.3 ppm (48h). After each ozone treatment, the fruit was cold stored at 0°C, 95% RH for 28 days as per the shipping protocol for most export markets. The fruit were then exposed to ambient temperature (25 °C) for 7 days. The sampling was done at 14-day intervals.

3.2.3 Decay rate

Decay rate was quantified using the methodology outlined by Yang et al. (2019). According to this approach, the number of decayed fruits per replicate is divided by the total number of fruits, and this quotient is then multiplied by 100 to express the result as a percentage as shown in equation 1.

$$\text{Decay rate} = \frac{Number\ of\ decayed\ fruits}{Total\ number\ of\ fruits}\ x\ 100 \qquad \text{Eq. 1}$$

3.2.4 Mass loss

Like many other fresh fruits, lemons are often sold on mass bases. Storage conditions and treatments can have a positive or negative impact on fruit mass. The measurement of fruit mass was conducted using a calibrated electronic weighing scale (RADWAG Wagi Electronic Inc., Poland). Lemons were weighed upon arrival at the laboratory (Day zero) and at the end of each storage interval, employing a separate sample of five replicates for each treatment condition. The calculation of mass loss was performed using the following equation established by Akhtar et al. (2010) in equation 2

$$\text{Mass loss (\%)} = \frac{Mi-Mf}{Mi}\ x\ 100$$

Where, Mi = Initial mass

Mf = Final mass Eq. 2

3.2.5 Fruit Colour

Colour is one of the key parameters used by consumer when purchasing fruit. Postharvest treatments have been demonstrated to affect fruit change during storage. In this study, fruit colour was objectively quantified based on L*, a*, b* and h parameters using CR 400 Chromameter (Minolta Co. Ltd., Osaka, Japan). Colour was measured from three random spots on the equatorial position of 6Y = 91.59, X = 0.3167 and y = 0.3315. The total colour difference was expressed as a citrus colour index (CCI) according to Vidal *et al.* (2013).

$$CCI = \frac{1000 \text{ x a}}{\text{L x b}} \qquad \text{Eq. 3}$$

3.2.5 Fruit firmness

Fruit firmness was quantified according to the protocol established by González-Aguilar et al. (2001), utilizing a handheld penetrometer (Bareiss, Germany) equipped with an 8 mm plunger tip. The firmness measurements were taken on opposite cheeks of each fruit, and the resulting data were expressed in newtons (N).

3.2.6 Statistical Analysis

The collected data were analyzed using analysis of variance (ANOVA) with the GenStat statistical software (GenStat 23rd Edition, VSN International Ltd, UK). Mean separation was conducted employing Tukey's least significant difference method at a significance level of 5%.

3.3 Results and Discussion

3.3.1 Decay rate

The interaction between treatment and storage time had no significant ($p > 0.05$) effect on decay rate of 'Eureka' lemons. Similarly, storage duration has no significant effect on decay incidence. However, gaseous ozone treatment significantly ($p < 0.001$) affected the decay incidence of the fruit (Figure 3.1 and Figure 3.2). The untreated fruit showed a relatively low decay incidence, while the treated fruits showed a high decay rate according to the intensity of the treatment, with O_3(48h) showing the highest decay rate.

Untreated fruit started showing signs for decay at Day 35, while for O_3 treated fruits the decay in fruits started showing at day 28 with O_3(48h) exhibiting the highest decay increase of 4% from Day 28 to Day 35. The O_3(24h) showed 3.2 % increase with the control being the lowest by 0.91%. The findings of the current study align with those of Cao et al. (2022) who found that 'Guichang' kiwi fruit treated with 300 µL/L for 3h, stored at 1°C and 85 ± 5% RH for 120 days had the highest decay incidence compared to those treated with 100 μL/L and 200 μL/L of ozone. Notably, although both ozone treatments had decay incidence, the level was 2% lower in O_3(24h) compared to O_3(48h) ozone treatment. This reveals that high ozone concentrations might compromise the cellular tissues and membrane integrity of the treated fruit thereby causing decay. However, our findings are in contrast with those of Nayak et al. (2020) where the 'Winter Dawn' strawberry fruits treated with 0.1 ppm aqueous ozone for 2 minutes stored at ambient (25 ± 2 °C and 45–50% RH) resulted in 22% decrease as compared to the 44% decrease of the untreated fruits.

Ozone changes the microbial community on the surface of the fruit, this could be another factor contributing to the decay incidence. While it may reduce some microorganisms, it can also allow the proliferation of the ozone resistant organisms which makes the fruit vulnerable to decay (Zhang et al., 2022). Lemons are known to have a low pH (acidic) as compared to the other fruits. The low pH is instrumental in delaying the onset of decay incidence (Serna-Escolano et al., 2021). Panou et al. (2021) observed in their study that the fresh strawberries (*Carbarosa*) treated with 0.5 ppm and 1.0 ppm stored at 1°C, 90% RH showed an increase in pH and thereby causing decay (Yakoby 2000; Ajayi & Olasehinde, 2009). The concentration of the gaseous ozone applied, and the period are an important factor to the decay of the fruit.

Figure 3.10: The severity of decay on the 'Eureka' lemon fruit at different concentrations **A** (Control), **B** (O_3 24h), **C** (O_3 48h) and stored at 0°C, 95% RH for twenty-eight days and shelf-life for seven days.

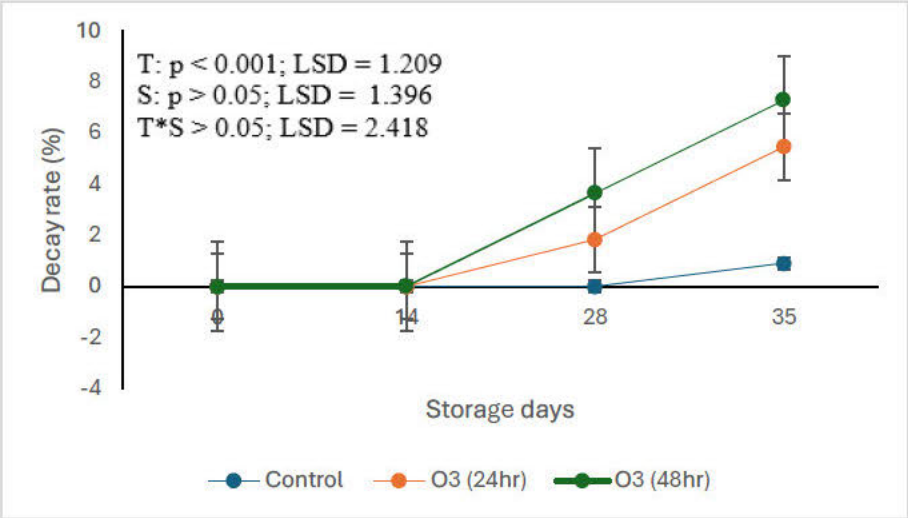

Figure 3.11:Effect of gaseous ozone on decay rate of 'Eureka' lemons during cold storage at 0°C for twenty-eight days and shelf-life at ambient temperature for seven days (±SE, n=5)

3.3.2 Fruit mass loss

The mass loss in 'Eureka' lemons varied among the treatments in relation to the storage days (Figure 3.3). Statistically, the treatments as well as the interaction between the treatments and storage duration did not significantly ($p > 0.05$) affect the mass loss of the lemon fruit. On the other hand, the storage duration significantly ($p < 0.05$) affected the fruit mass loss. For all the treatments, mass loss sharply increased during storage (Figure 3.3). Although there were no statistical differences between the treatments, ozonated fruit appeared to have lower mass loss compared to the control treatment. For instance, at the end of the storage days the control had 16% loss which was higher than the O_3(24h) and O_3(48h) treatments which had 10% and 9% loss, respectively.

The findings of the present study are consistent with those reported by Li et al. (2022), which demonstrated reduced mass loss in fresh-cut red pitaya subjected to ozone treatment at concentrations of 2, 4, and 6 mg/L for 20 minutes over an eight-day storage period, compared to untreated samples. Several factors have been identified that contribute to the beneficial effects of gaseous ozone in mitigating weight loss in fresh produce. Specifically, Li et al. (2022) argued that ozone treatment activates the antioxidant defence mechanisms, facilitating the metabolism of reactive oxygen species (ROS). This activation results in the upregulation of key enzymes such as superoxide dismutase (SOD) and peroxidase, which play critical roles in neutralizing ROS and maintaining cellular integrity in fruits during storage.

The preserved cellular integrity prevents the leakage of water from the fruit lenticels. Shen et al. (2021) in their study observed that 'Red Delicious' apples treated with gaseous ozone (60.2 ± 5.7 or 78.7 ± 13.2 µg/L) subjected to the refrigerated air (RA; 0.2 ± 0.1◦C) and CA (0.2 ± 0.1◦C, 3.1 ± 0.0% O2, 0.4 ± 0.0% CO2) with 90 % RH for 36 weeks, had more closed lenticels than the controlled apples. In fact, Li et al. (2022) hypothesized that ozone can enhance the cuticle's integrity which serves as a blockage to water loss.

Another reason for lower mass loss in ozonated fruit could be linked to the ability of ozone to instantly react with ethylene, which is the hormone that causes ripening and senescence in fruits (Liu et al., 2021). Thus, ozone inhibits ethylene production, delays ripening and slow down mass loss which is associated with increased metabolic activity.

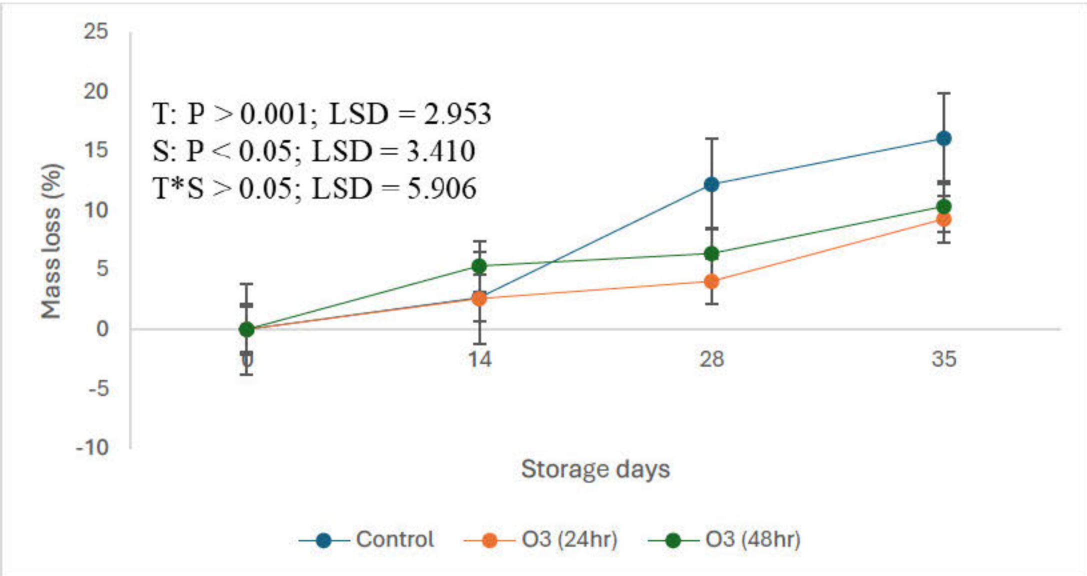

Figure 3.12: Effect of gaseous ozone on mass loss of 'Eureka' lemons during cold storage at 0°C for twenty-eight days and shelf-life at ambient temperature for seven days (±SE, n=5)

3.3.3 Colour

Colour is a parameter that attracts consumers to the product and lemons are mostly characterised by a yellow colour. The interaction between treatment and the storage did not significantly ($p > 0.05$) affect the colour of the fruit. Similarly, the treatment did not significantly ($p > 0.05$) affect colour changes, whereas the storage significantly ($p < 0.05$) influenced fruit colour.

The untreated fruit showed variations in the colour as the values fluctuated from the beginning until the end of the storage (Figure 3.4). At the beginning of the storage (Day 0) the colour was

-1.29 with a huge decline of 57% from Day 14 to Day 28. The O_3(24h) treatment showed an increase with no decline from the beginning of the storage to the end of the storage. The same trend followed with O_3(48h) from Day 0 to Day 28. However, from day 28 there was decline of 27%. From Day 0 to Day 35, the control showed an increase of 43%, O_3(24h) showed an increase of 52% while the O_3(48h) showed the biggest increase of 54%.

On Day 14, the O_3(24h) treatment showed the highest value of L as compared to the other treatments. The O_3(48h) showed a high value in all storage days for a* as compared to the other treatments with the highest value being at Day 28. Thereafter, a slight decline was observed at Day 35 during room temperature storage. Our findings correlate with Bambalele et al. (2023) who reported higher chroma values in 'Keitt' mango fruit exposed to different durations of ozone treatment. This indicates that exposure time and storage duration is critical when developing protocols for ozonated storage conditions.

Biochemical mechanisms coordinate the changes in fruit coloration. Ozone exposure has been shown to enhance the levels of antioxidants in fruits, notably phenolic compounds and carotenoids, which are crucial for the pigmentation of lemon fruits (Li et al. 2022). The oxidative stress induced by ozone can stimulate the synthesis of secondary metabolites that significantly contribute to fruit pigmentation (Li et al., 2022). Although the observed differences were not statistically significant, our findings suggest that O_3(48h) treatment is effective in maintaining the colour integrity of 'Eureka' lemon fruits.

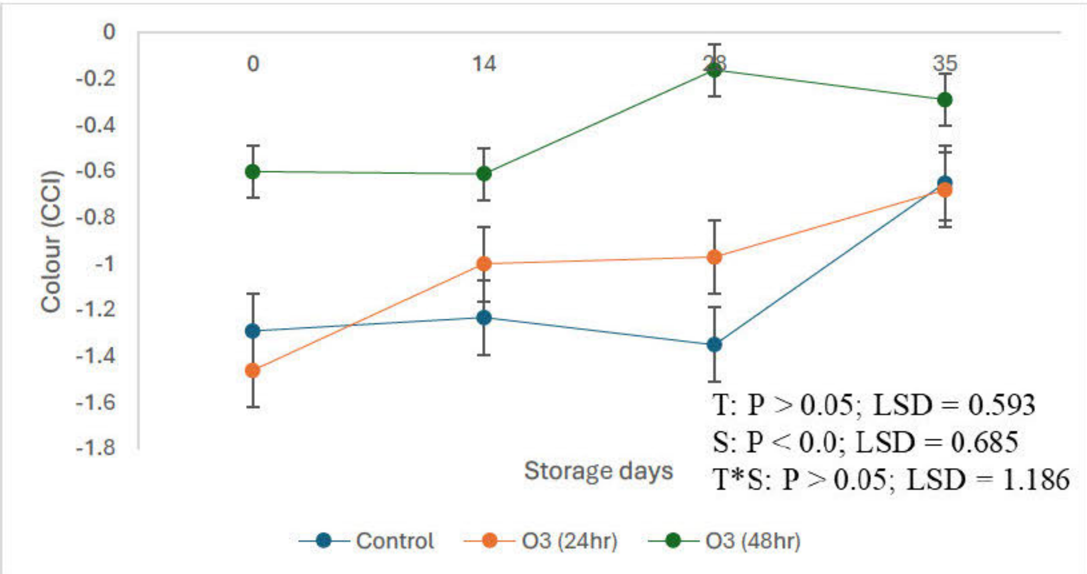

Figure 3.13: Effect of gaseous ozone on colour (CCI) of 'Eureka' lemons during cold storage at 0°C for twenty-eight days and shelf-life at ambient temperature for seven days (±SE, n=5)

3.3.4 Firmness

Firmness is one of the key quality parameters used by the consumers to assess fruit suitability. In this study, the interaction between treatment and the storage duration did not significantly ($p > 0.05$) affect fruit firmness. Notably, the treatments significantly ($p < 0.001$) affected the firmness level during storage. For all the ozone treatments, there was a significant decline in fruit firmness (Figure 3.5).

The study demonstrated that 'Eureka' lemon fruits subjected to O_3(48h) treatment exhibited a reduced firmness loss of 1.79% compared to the control group and O_3(24h) treatment, which recorded losses of 2.58% and 2.60%, respectively. These findings align with those reported by Bambalele et al. (2023), who observed that a 0.25 mg/L ozone treatment preserved mango fruit firmness for up to 14 days. Similarly, Zhang et al. (2021) found that a 1 mg/L ozone treatment for 5 h effectively maintained the firmness of cantaloupe melons stored at 4 °C for 30 days. The preservation of firmness in these fruits is likely due to ozone's capacity to inhibit the activity of cell wall degrading enzymes, such as polygalacturonase and β-galactosidase (Bambalele et al., 2023).

Fruit firmness is fundamentally dependent on the structural integrity of the cell wall, which consists of polysaccharides such as pectin, cellulose, hemicellulose, and lignin, all of which contribute to its strength (Mthembu et al., 2024). The enzymatic degradation and solubilization of cell wall polysaccharides (CWPs) result in modifications to the cell wall's structure and composition, leading to textural changes that manifest as fruit softening (Mthembu et al., 2024). Loss of firmness in fruit may be attributed to alterations in the structural components of the cell wall, turgor pressure variations, and changes in intercellular adhesion (Fraeye et al., 2009). Additionally, gradual cellular degradation during storage due to senescence further exacerbates the loss of fruit firmness (Kumar et al., 2018).

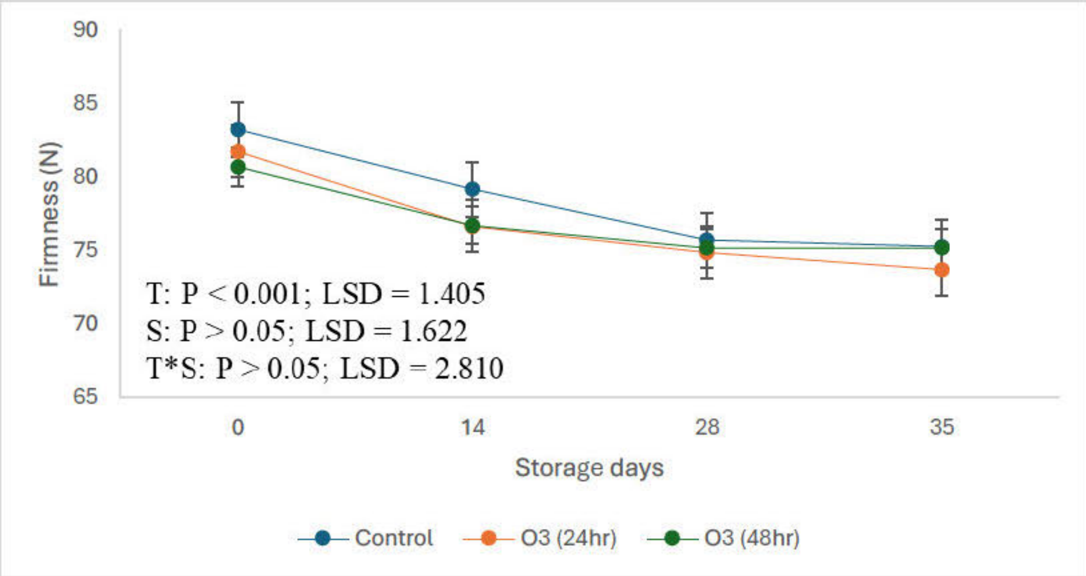

Figure 3.14: Effect of gaseous ozone on firmness loss of 'Eureka' lemons during cold storage at 0°C for twenty-eight days and shelf-life at ambient temperature for seven days (±SE, n=5)

3.4 Conclusion

This study demonstrated that gaseous ozone effectively inhibited mass loss and firmness reduction while promoting color development in 'Eureka' lemon fruits. However, it was observed that the incidence of decay in ozone-treated fruits was higher compared to untreated controls. This phenomenon may be attributed to ozone's alteration of the microbial community on the fruit's surface, potentially allowing for the proliferation of ozone-resistant microorganisms. Further research is warranted to investigate the specific decay-causing bacteria and fungi, as well as the effective concentrations of ozone required to mitigate these issues. Both ozone treatments (24 h and 48 h) significantly reduced weight loss in 'Eureka' lemon fruits compared to the control group. However, extending the exposure time from 24 h to 48 h may lead to increased mass loss over time. In terms of firmness and color retention, the 48-h ozone treatment proved to be the most effective. Nonetheless, additional research is necessary to explore the effects of ozone on the physical parameters of 'Eureka' lemons, particularly regarding cell wall degrading enzymes that are crucial for maintaining fruit firmness.

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Chapter 4

The effect of gaseous ozone in antioxidant and biochemical attributes of ‘Eureka’ lemons

Abstract

Gaseous ozone (O_3) is increasingly recognized as an effective postharvest treatment for preserving the quality and extending the shelf-life of fresh horticultural products. This study examined the impact of gaseous ozone on the antioxidant and biochemical properties of ‘Eureka’ lemons. The fruits were harvested and stored at 0 °C with 95% relative humidity for a duration of 28 days, then subsequently stored at ambient temperature for 7 days. The lemons were exposed to 0.3 ppm of gaseous ozone for 24 h or 48 h, with untreated lemons serving as the control group. The study evaluated changes in total phenolics, flavonoids, DPPH radical scavenging activity, carotenoids, condensed tannins, and ascorbic acid at 14-day intervals. The results indicated that gaseous ozone significantly ($p < 0.05$) preserved the antioxidant capacity and biochemical attributes of the lemons. The effects of ozone on antioxidants varied, the O_3(24h) treatment positively influenced phenolic content, which increased from 148.8 g/kg GAE at Day 0 to 187.2 g/kg GAE by Day 35. In contrast, the O_3(48h) ozone treatment significantly enhanced carotenoid levels, increasing from 5.718 µm TE/g at Day 0 to 6.343 µm TE/g at the end of storage period. These findings suggest that gaseous ozone not only effectively extends the shelf-life of treated produce but also helps retain its biochemical and nutritional composition during storage.

Keywords: Gaseous ozone, citrus fruit, post-harvest treatments, antioxidants, quality, shelf-life.

4.1 Introduction

Citrus fruits hold significant socio-economic and cultural importance globally, making them among the most widely consumed fresh fruits (Iglesias et al., 2007). Among the various citrus species, lemon ranks as one of the most crucial, following orange and mandarin (Perez-Perez, 2005). The distinct flavour and acidity of lemons contribute to their status as a value-added product, leading to extensive use in industrial applications (Park et al., 2022).

Lemons are rich in antioxidants and bioactive compounds, including vitamins, minerals, essential oils, phenolics, and carotenoids (González-Molina et al., 2010). Notably, vitamin C is one of the most prevalent biochemical compounds found in lemons (Park et al., 2022). Lavelli et al. (2009) reported that vitamin C accounts for approximately 65% of the antioxidant and antiradical activity present in fruits. Due to its sensitivity to heat, careful temperature regulation during storage is essential to preserve vitamin C levels (Mditshwa et al., 2017).

Carotenoids, which include β-carotene as the most prominent pigment, are responsible for the yellow coloration in lemons (Olson & Krinsky, 1995). These pigments function as antioxidants in animals, and diets rich in carotenoids are associated with reduced risks of various diseases, including cancer and cardiovascular disorders (Mayne, 1996). Carotenoids are synthesized not only by photosynthetic organisms but also by non-photosynthetic ones (Meena et al., 2013). In lemons, carotenoids play a critical role in protecting the photosynthetic apparatus from reactive oxygen species (ROS), particularly singlet oxygen (Meena et al., 2013).

Several postharvest factors significantly influence the antioxidant capacity of 'Eureka' lemons. Cold storage has been shown to reduce the total antioxidant content of citrus fruits, while postharvest treatment with ethephon can enhance antioxidant levels (Sun et al., 2019). Other postharvest factors such as handling practices, treatments, packaging methods, and marketing strategies also impact the antioxidant capacity of lemons (Zhang & Zhou, 2019). Current postharvest treatments are known to effectively maintain the antioxidant capacity of fresh produce. For instance, Eskandari et al. (2014) demonstrated that Arabic gum treatment increased vitamin C content compared to control samples. However, certain coating treatments have been found detrimental to consumer health (Mditshwa et al., 2017), and high-temperature heat treatments have proven ineffective due to potential heat damage that adversely affects cell walls (Mditshwa et al., 2017).

There is a growing demand for alternative treatments to mitigate fruit deterioration while ensuring food safety. Gaseous ozone has emerged as a promising postharvest treatment for citrus fruits, offering numerous benefits for quality preservation. The application of gaseous ozone is associated with reduced ethylene levels, thereby delaying ripening and extending shelf-life (Bambalele et al., 2023). As a potent disinfectant and sanitizing agent with a relatively short lifespan, gaseous ozone contributes to food safety for consumers (Shezi et al., 2020). Nevertheless, literature on the impact of ozone on citrus fruits remains limited. A study by Zhu et al. (2019) indicated that ozone treatment enhances antioxidant activity, total phenolic content, flavonoid levels, and prolongs the shelf-life of 'Satsuma' mandarins. Although gaseous ozone has been reported effective in extending shelf-life and preserving biochemical properties across various fresh horticultural products, its specific effects on the antioxidant capacity of 'Eureka' lemons have yet to be explored. Consequently, this study aims to evaluate the impact of short-term gaseous ozone treatments on the antioxidant capacity of 'Eureka' lemons.

4.2 Materials & method

4.1.1 Fruit material

'Eureka' lemon was collected at Carisbrooke Citrus Valley Farm, Ixopo, KwaZulu-Natal (30.24172° S, 30.01315° E). Upon arrival at the Postharvest Laboratory of the Department of Horticultural Science, fruit were graded for uniformity according to colour and size, and thereafter stored in carton boxes at 0 °C and 95% relative humidity (RH) for 28 days as per the shipping for most export markets. The fruit were then exposed to ambient temperature (25 °C) for 7 days.

4.1.2 Ozone treatment

The fruit were treated with 0.3 ppm gaseous ozone equipped with an ozone analyzer (Corona discharge ozone generator, Ozone Purification Technology, Johannesburg, South Africa). Fruits were assigned to respective gaseous ozone treatments where time was the treatment factor: T1: Control (untreated lemons); T2: 0.3 ppm (24h); T3: 0.3 ppm (48h). After each ozone treatment the fruit was exposed to cold storage with 0°C with 95% RH.

4.1.3 Sample preparation

The experiment was arranged in a complete randomised design, each treatment had 5 replicates with each replicate containing 22 fruits. The sampling was done at 14-day intervals. On each sampling day (D_0, D_{14}, D_{28} and D_{35}), the portion of the flavedo (peel) was cut from the fruit and stored at -22°C for later use. For the sampling powder, the flavedo was then freeze dried. The sample extraction procedure varied from the parameters according to the prescribed method.

4.1.4 Quantification of total phenolics

The total phenolic content was quantified using a spectrophotometric method as outlined by Ilahy et al. (2011), with specific modifications. In this procedure, 0.155 g of the sample was extracted using 3 mL of 80% methanol. The mixture was subjected to vortexing for 1 minute, followed by centrifugation for 10 minutes utilizing a GenVac centrifuge (SP Scientific, Genevac LTD., Suffolk, UK). To assess the total phenolic content, 100 µL of the supernatant was transferred to a test tube and diluted with 950 µL of distilled water. Subsequently, 125 µL of Folin–Ciocalteu reagent was added. After a reaction period of 3 minutes, 1250 µL of a 7% sodium carbonate solution was introduced, and distilled water was added to achieve a final volume of 4 mL. The resulting solution was incubated in darkness at room temperature for 90 minutes. Absorbance measurements were taken at 760 nm against a blank, and the results were expressed as grams of gallic acid equivalent per kilogram (g/kg GAE).

4.1.5 Quantifying the flavonoids

The total flavonoid concentration was determined using the aluminum chloride colorimetric assay as described by Kamtekar et al. (2014). In this method, 0.2 g of the sample was extracted with 100% ethanol. Following extraction, 500 µL of the diluted solution was combined with 4 mL of distilled water and 300 µL of a 5% sodium nitrite solution. This mixture was allowed to stand for 5 minutes, after which 300 µL of a 10% aluminum chloride solution was added. After an additional 6 minutes, 2 mL of a 1 M sodium hydroxide solution was introduced. Distilled water was then added to achieve a final volume of 10 mL, resulting in a color change from orange to yellowish. The absorbance of the solution was measured at 510 nm using a spectrophotometer against a blank control. All samples were analyzed in triplicate, and the results for flavonoids were expressed as grams of quercetin equivalents per kilogram of dry mass (g/kg QE).

4.1.6 Determination of total carotenoids

Total carotenoids were quantified using the method established by Lichtenthaler (1987). In this procedure, 0.2 g of flavedo tissue was ground in 2 mL of 80% (v/v) acetone and subsequently centrifuged at 3000 × g for 10 minutes at 4°C. To ensure optimal detection of carotenoids, as well as chlorophyll a and b, the absorbance of the supernatant was measured at wavelengths of 470 nm, 646.8 nm, and 663.2 nm. These absorbance readings facilitated the calculation of the concentrations of total carotenoids, chlorophyll a, and chlorophyll b according to the following equations:

$$Ca = 12.25\, A663.2 - 2.79\, A646.8 \qquad \text{Equation 1}$$

$$Cb = 21.50\, A646.8 - 5.10\, A663.2 \qquad \text{Equation 2}$$

Cx+c = (1000 A470 - 1.82 Ca -85.02 Cb) / 198, where Ca represents chlorophyll a, Cb chlorophyll b and Cx+c total carotenoids Equation 3

4.1.7 Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH assay was conducted following the methodology outlined by Thaipong et al. (2006). A stock solution was prepared by dissolving 0.024 g of DPPH in 100 mL of 100% methanol (v/v). The working solution was created by mixing 10 mL of the stock solution with 45 mL of 100% methanol (v/v), achieving an absorbance of 1.1 ± 0.02 units at 515 nm. An antioxidant extract (150 µL) was then allowed to react with the DPPH solution (2850 µL) for 24 hours in the dark. The absorbance was measured at 515 nm. A standard curve demonstrated linearity between concentrations of 25 and 800 µM Trolox. The results were expressed in micromoles of Trolox equivalents per gram of dry mass (µM TE g^{-1} DM).

4.1.8 Extraction and determination of rind ascorbic acid

Ascorbic acid was extracted from the freeze-dried sample using a method adapted from Hernández et al. (2006) for citrus applications. In brief, 150 ± 0.5 mg of the dry sample was combined with 5 mL of 3% (w/v) aqueous metaphosphoric acid. The mixture was homogenized for 1 minute using a vortex mixer and then placed on ice for 5 minutes. Following this, the sample was centrifuged for 20 minutes using a GeneVac centrifuge (SP Scientific, Genevac LTD., Suffolk, UK) with the lamp turned off. Subsequently, 0.5 mL of the supernatant was incubated in the dark with 2.5 mL of 2,6-dichloroindophenol dye (prepared by dissolving 0.015 g of dye in 100 mL of distilled water). Absorbance readings were taken at 515 nm in triplicate, and the results were expressed as grams per kilogram on a dry weight basis (g/kg).

4.1.9 Condensed Tannins (CT)

Condensed tannins were quantified using a modified method based on Porter et al. (1986). In this procedure, 200 mg of flavedo powder was extracted with 70% acetone. Following extraction, 0.5 mL of the extract was combined with 3.0 mL of butanol-HCl reagent and 100 µL of ferric reagent, and the mixture was vortexed thoroughly. The test tubes were then covered and subjected to heating at 97°C for 60 minutes. After cooling, the absorbance of the solution was measured at 550 nm. The concentration of condensed tannins, expressed as a percentage of dry matter, was calculated using the formula: (A 550 nm x 78.26 x Dilution factor*) / (% dry matter).

4.1.10 Statistical analysis

The collected data were analyzed using analysis of variance (ANOVA) with GenStat statistical software (GenStat 23rd Edition, VSN International Ltd, UK). Mean separation was conducted using Tukey's least significant difference (LSD) method at a 5% significance level. Additionally, principal component analysis (PCA) was performed utilizing XLSTAT 2024.

4.3 Results and discussion

4.3.1 Total phenols

Phenolic compounds represent a diverse group of secondary metabolites synthesized during the metabolic processes of plants (Rahman et al., 2022). In citrus fruits such as lemons, these compounds significantly influence sensory attributes and contribute to the antioxidant capacity of the fruit (Xi et al., 2017; Haida et al., 2022; Mcharek & Hanchi, 2017). The interaction between storage conditions and treatment methods was found to have a statistically significant ($p < 0.05$) effect on the total phenolic content in 'Eureka' lemons. While storage conditions significantly ($p < 0.05$) influenced phenolic levels, the treatments applied did not yield a significant ($p > 0.05$) impact on the phenolic compounds.

The O_3(24h) treatment resulted in an increased concentration of phenolic compounds from Day 0 to Day 35 (Fig. 1). Shezi et al. (2020) suggest that this elevation in phenolic compounds may be linked to the activity of phenylalanine ammonia-lyase (PAL). The activation of the phenylpropanoid pathway, which is initiated by PAL, is known to contribute to the accumulation of phenolic compounds (Gonzalez-Mendoza et al., 2018). This increase in phenolic content serves as a non-enzymatic defence mechanism employed by plants to counteract oxidative stress induced by reactive radicals.

Balawejder et al. (2021) conducted an investigation into the effects of gaseous ozone on the polyphenolic content in apples, revealing that exposure to ozone for durations ranging from 1 to 30 minutes positively influenced polyphenol levels. Their findings indicate that the oxidative stress induced by ozone may stimulate the synthesis of polyphenols as a defensive mechanism in plants. Supporting this, Vettraino et al. (2019) observed a 40% increase in phenolic content after 90 days of storing chestnuts compared to control samples. The current research demonstrates that a 24-h treatment with ozone enhances the phenolic content in 'Eureka' lemons, suggesting that this treatment moderately activates phenolic compounds, thereby aiding the fruit's defence against oxidative stress from free radicals.

Increased levels of phenolic compounds significantly enhance the antioxidant capacity of fruit, particularly lemons. These compounds play a crucial role in neutralizing free radicals and mitigating oxidative stress, as evidenced by findings from Shimizu et al. (2019). Furthermore, phenolic compounds exhibit various biological effects, including antimicrobial and anti-inflammatory properties, as reported by Singh et al. (2020). Importantly, phenolics are recognized as vital phytochemicals that contribute to the nutritional and functional attributes of fruit, thereby increasing their overall nutritional value (Papoutsis et al., 2016). The enhancement of these attributes is associated with improved shelf-life in lemons.

The phenolic content observed under both control conditions and O_3(48h) treatment exhibited a similar declining trend throughout the storage period (Fig. 4.1). This reduction in phenolic compounds may be attributed to the stress induced by cold storage temperatures. Additionally, exposure to elevated concentrations or doses of gaseous ozone can lead to a decrease in phenolic levels due to over-oxidation, given ozone's strong oxidizing potential (Modesti et al., 2021). This phenomenon helps to elucidate the observed decline in phenolic compounds following O_3(48h) exposure.

The reduction in phenolic compounds can significantly impact the quality and nutritional value of fruit. Phenolics are essential for enhancing antioxidant capacity and improving the visual appeal of fruit, thereby contributing to its health benefits (Eroğul et al., 2023). During storage, fruits typically experience a decline in these compounds unless treated with specific interventions, which accounts for the observed decrease in the control samples. This decline adversely affects the fruit's ability to combat oxidative stress and maintain freshness (Pott et al., 2020), ultimately influencing its flavour, colour, and aroma. Consequently, a decrease in phenolic content compromises both the antioxidant capacity and nutritional value of the fruit.

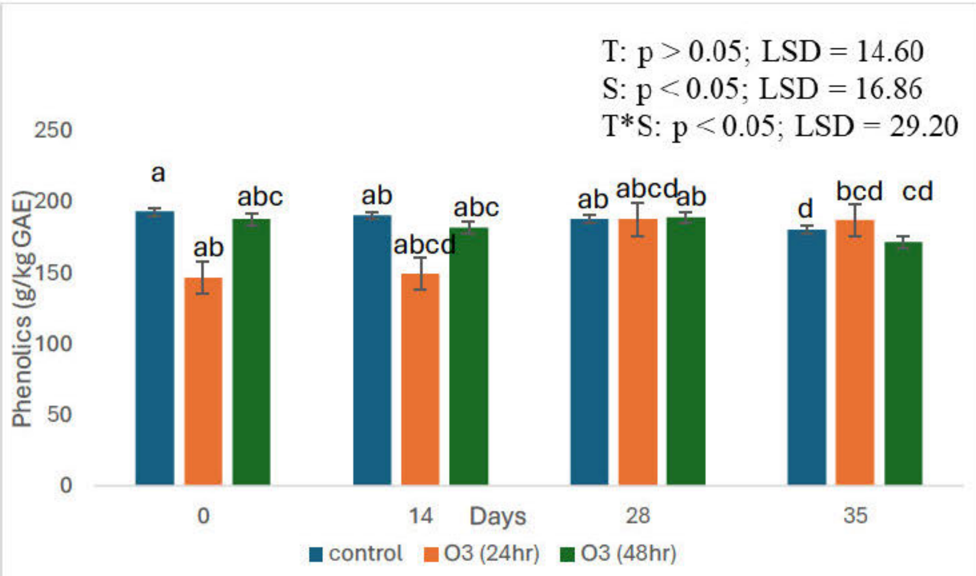


Figure 4.15: Effect of gaseous ozone on phenolics of 'Eureka' lemon peel during cold storage at 0°C for twenty-eight days and shelf-life at ambient temperature for seven days (±SE, n=5)

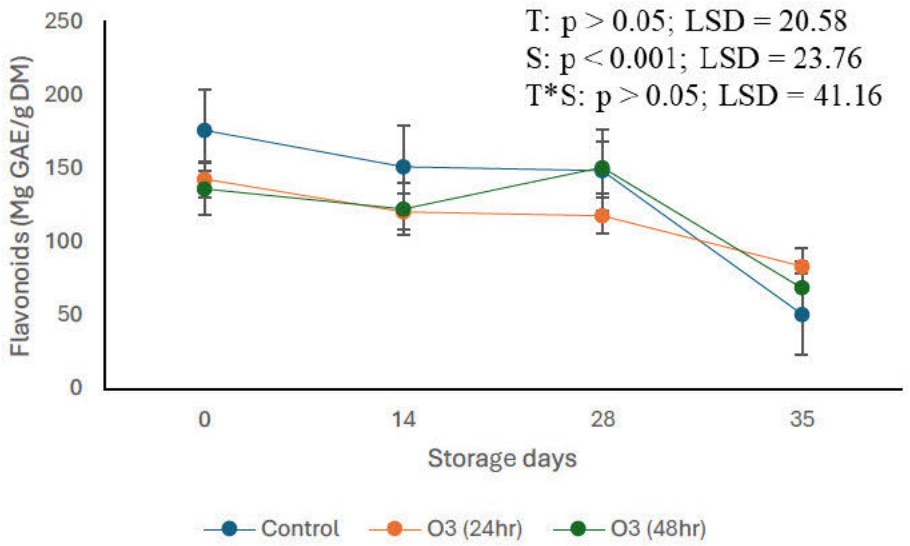
4.3.2 Flavonoids

Flavonoids play a significant role in the health benefits and essential characteristics of lemons, including their appearance, flavor, and nutritional value (Gattuso et al., 2007). This group of compounds encompasses flavonones, such as eriocitrin, as well as flavones like diosmin, diosmetin, and apigenin di-glucoside (Gattuso et al., 2007; Barreca et al., 2020). Flavonoids are secondary metabolites synthesized through the shikimate pathway and phenylpropanoid metabolism (Stafford, 1990).

Throughout the storage period (Days 0, 14, 28, and 35) and across all treatments (control, O_3 for 24 h, and O_3 for 48 h), a gradual decline in flavonoid content was observed (Fig. 4.2). Statistical analysis indicated that storage significantly affected flavonoid levels ($p < 0.001$). However, the application of gaseous ozone treatments did not significantly ($p > 0.05$) alter flavonoid content. Furthermore, the interaction between storage duration and treatment type did not have a significant ($p > 0.05$) effect on flavonoid levels.

The decline in flavonoids during storage can be attributed to several factors. A primary cause of flavonoid degradation is the alteration in enzyme activity and the reduction of astringency that occurs during the ripening process, leading to phenolic degradation (Ma et al., 2019). Additionally, the generation of free radicals during storage significantly contributes to the reduction of flavonoids, as this process involves the oxidation of phenolic compounds, resulting in decreased flavonoid levels (Malik et al., 2022).

In a study examining the effects of gaseous ozone on the postharvest quality of 'Satsuma' mandarins, Zhu et al. (2019) reported a slight increase in flavonoid content following ozone treatment. This increase was linked to the activation of several intercellular enzymes, including chalcone isomerase (CHI), β-1,3-glucanase (GLU), chitinase (CHT), phenylalanine ammonia-lyase (PAL), and peroxidase (POD). The fluctuations in flavonoid levels may be influenced by various factors such as storage temperatures, exposure to light, and oxygen levels (Pott et al., 2020). These changes have the potential to cause fluctuations and degradation in flavonoid levels. The physiological changes in fruit during storage can impact the stability of flavonoids, contributing to their reduction over time.



"Figure 4.16: Effect of gaseous ozone on flavonoids of 'Eureka' lemon peel during cold storage at 0°C for twenty-eight days and shelf-life at ambient temperature for seven days (±SE, n=5)

4.3.3 Carotenoids

In lemons, carotenoids are responsible for the characteristic yellow pigmentation (Lana et al., 2020). These compounds are classified into two categories: carotenes, which consist solely of carbon and hydrogen atoms, and xanthophylls, which contain oxygen in addition to carbon and hydrogen. The current study found that the carotenoid content was significantly influenced by the interaction between storage duration and ozone treatment ($p < 0.05$). Throughout the storage period (from Day 0 to Day 28), carotenoid levels exhibited an upward trend (Fig. 4.3). However, at the end of shelf-life storage (Day 35), there was a slight decrease in carotenoid content across all treatments (Fig. 4.3).

Ozonation can have diverse effects on carotenoid levels in fruits. Some studies indicate that exposure to gaseous ozone may lead to a decrease in carotenoid content during storage. For example, Botondi et al. (2015) reported a reduction in β-carotene and lycopene levels in wine grapes subjected to high concentrations of ozone for prolonged periods, which may trigger oxidative cleavage of carotenoids and result in the production of abscisic acid (ABA). Conversely, Bambalele et al. (2023) found that ozone treatment increased carotenoid content in mango fruit during storage, highlighting the variability in response depending on the type of fruit and treatment conditions.

The current study aligns with the findings of Bambalele et al. (2023), who also reported an increase in carotenoid levels in ozonated mango fruit during storage. Ozone has the ability to bind to the double bonds of carotenoid molecules, leading to their oxidation, which can affect the tissue color (Botondi et al., 2021). For instance, Goffi et al. (2019) demonstrated that gaseous ozone positively influenced carotenoid content in 'Soreli' kiwifruit. Specifically, storage at 2°C under a treatment of 300 ppb gaseous ozone improved the quality of yellow-fleshed fruit, while storage at 4°C did not preserve color effectively. The impact of gaseous ozone on carotenoids varies depending on both the duration of exposure and the length of storage.

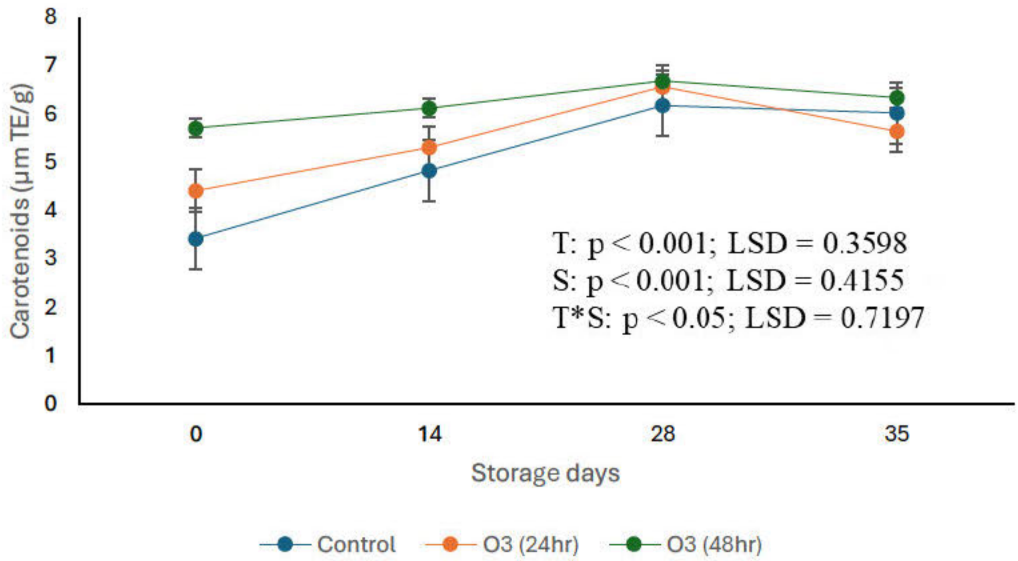


Figure 4.17: Effect of gaseous ozone on carotenoids of 'Eureka' lemon peel during cold storage at 0°C for twenty-eight days and shelf-life at ambient temperature for seven days (±SE, n=5)

4.3.4 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The findings indicated that both the storage duration and the interaction between storage duration and treatment had an insignificant effect on DPPH scavenging activity ($p > 0.05$). However, the treatment itself had a highly significant effect on DPPH activity ($p < 0.001$). Throughout the storage period, both the control and O_3(48h) treatments exhibited an upward trend in DPPH activity (Fig. 4.4). In contrast, the O_3(24h) treatment showed a decrease in DPPH activity from day 14 to day 28, after which it peaked, ultimately surpassing the levels observed in the other two treatments (Fig. 4.4).

The current study aligns with the findings of Zhu et al. (2019), which demonstrated that the DPPH scavenging activity in 'Satsuma' mandarins increased over the storage period following gaseous ozone treatment. Specifically, Zhu et al. (2019) reported that maintaining a gaseous ozone concentration of 2.5 μg/L for 24 h significantly enhanced the DPPH scavenging activity in these mandarins. Furthermore, an increase in DPPH levels in lemons indicates a substantial capacity for free radical neutralization, as noted by García-Nicolás et al. (2023). This increase is particularly advantageous, given the established link between antioxidants and the mitigation of chronic diseases such as cancer, cardiovascular conditions, and neurodegenerative disorders (Fadda et al., 2014). The antioxidant potential of lemons is attributed to compounds such as flavonoids, limonoids, and carboxylic acids, which are actively involved in free radical scavenging, as assessed through the DPPH assay (García-Nicolás et al., 2023).

To date, literature examining the effects of gaseous ozone on DPPH scavenging activity remains limited. This study represents a pioneering effort to investigate the influence of gaseous ozone on DPPH scavenging activity in lemons. Lin et al. (2020) identified several critical factors that may contribute to a decline in DPPH activity in lemons, including the degradation of antioxidant compounds capable of scavenging DPPH radicals, oxidation of these antioxidants, volatilization losses, and structural changes induced by variations in time and temperature. Notably, this study observed a slight decline in DPPH levels at Day 28 following O_3(24h). Zhang et al. (2020) suggested that such declines may be linked to ozone's capacity to enhance antioxidant enzymes such as superoxide dismutase and catalase, which may influence overall antioxidant activity.

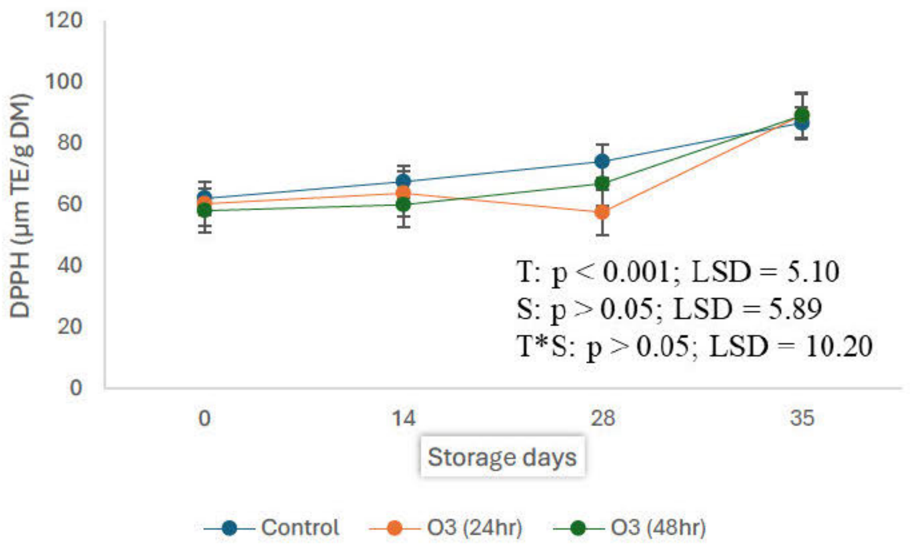


Figure 4.18: Effect of gaseous ozone on DPPH of 'Eureka' lemon peel during cold storage at 0°C for twenty-eight days and shelf-life at ambient temperature for seven days (±SE, n=5)

4.3.5 Ascorbic acid (AA)

Lemons are recognized as a significant source of ascorbic acid (AA), a water-soluble antioxidant that plays a crucial role in scavenging free radicals and protecting cells from oxidative stress and reactive oxygen species (ROS) generated during metabolic processes (Yimcharoen et al., 2019; Ferrada et al., 2020). The present study indicates that the interaction between storage duration and treatment significantly influences AA content, with statistical significance established at ($p < 0.001$). Initially, the control treatment exhibited elevated levels of AA; however, ozonated lemons demonstrated substantially higher AA concentrations compared to untreated lemons at the conclusion of the storage period (Figure 4.5).

These findings corroborate those of Miller et al. (2021), who reported elevated vitamin C levels in melon peel subjected to gaseous ozone treatment. Similarly, Onopiuk et al. (2017) observed comparable results in raspberries exposed to gaseous ozone at a concentration of 0.35 mg/L for 60 minutes over an eight-day storage period. The observed increase in ascorbic acid can be attributed to ozone's capacity to inhibit the activity of ascorbate peroxidase and ascorbate oxidase, enzymes that typically contribute to the degradation of ascorbic acid in various fruits (Liu et al., 2021).

Ascorbic acid enhances the fruit's resilience against oxidative damage induced by ozone exposure, suggesting that ozone stress may stimulate the biosynthesis of ascorbic acid from carbohydrate reserves within plant cells (Miller et al., 2021; Liu et al., 2021). The activation of antioxidative systems during ozone stress may elucidate the increase in ascorbic acid levels observed in treated lemons. However, it is noteworthy that high concentrations of gaseous ozone can lead to the oxidation of ascorbic acid. The treatments employed in this study were effective in preserving and enhancing ascorbic acid content.

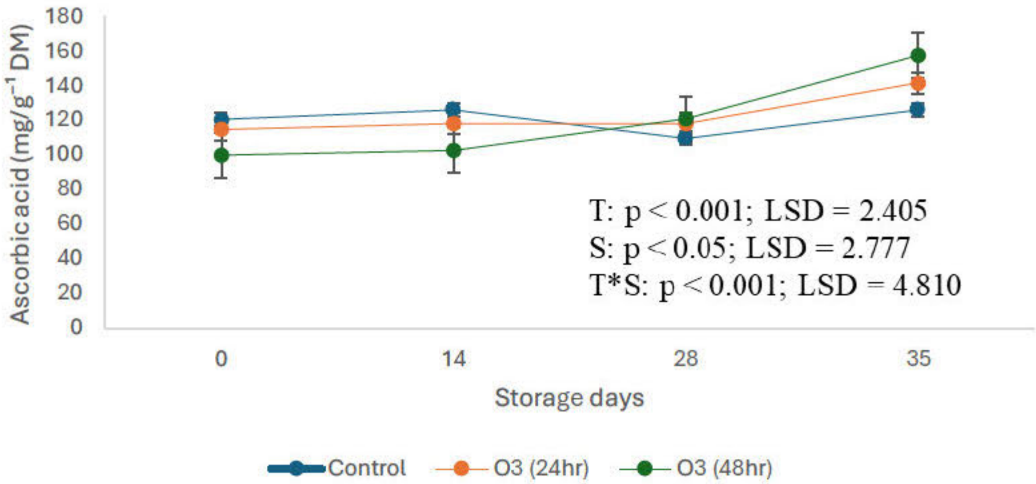


Figure 4.19: Effect of gaseous ozone on ascorbic acid of 'Eureka' lemon peel during cold storage at 0°C for twenty-eight days and shelf-life at ambient temperature for seven days (±SE, n=5)

4.3.6 Condensed tannins (CT)

Condensed tannins are a class of polyphenolic compounds found in many plants (Tong et al., 2021). They are formed by condensation of flavans which are a broader group of flavonoids and do not contain sugar residues. Condensed tannins have been studied for their importance in animals, acting as antimicrobial agents, and in humans by reducing blood pressure, promoting blood clotting, and modulating immune response (Chung et al., 1998). As much as condensed tannins possess these vital functions, they are however believed to be relatively low in lemons as compared to other plants. The current study assessed the influence of gaseous ozone on condensed tannins and is believed to be one of the few studies to be conducted on how the postharvest treatments affect condensed tannins.

The ozone treatment and storage days significantly ($p < 0.001$) affected the condensed tannins in lemons. In all treatments (Control, O_3 24h and O_3 48h) the highest levels of the condensed tannins were found on Day 14, which indicated a slight increase from Day 0 (Figure 4.6). An increase in condensed tannins is attributed to the plant's genotype, tissue development and environmental stress (Samtiya et al., 2020). In Day 14, there was no signs of tissue collapse in lemons, which makes sense to a positive response of the lemons the various ozone treatments the fruits were exposed to. Tissue collapse is a sign of a decay, in which the cell walls are slowly deteriorating and thus leading to a decrease of the various antioxidants in plants.

However, from Day 14 to Day 28 there was a huge decrease in condensed tannins as indicated in figure 6. The decrease could be attributed to the fruits slowly decaying due to the unfavourable conditions the fruits were exposed. A decrease in condensed tannins can be caused by the hydrolysis by enzymes and changes in chemical composition (Jylhä et al., 2021). This process is influenced by factors such as storage conditions, exposure to light and temperature variations (Panzella & Napolitano, 2022). This could explain the slight increase in CT from Day 28 to Day 35. Samtiya et al. (2020) highlighted that CT can also be activated by environmental stresses. Thus, since ozone is considered as a stress treatment, this could explain the increase of CT in towards the end of storage. Notably, the biggest increase at ambient storage was observed in O_3 48h, this indicates that the treatment caused enough stress to the fruit and thus causing an increase to the CT levels.

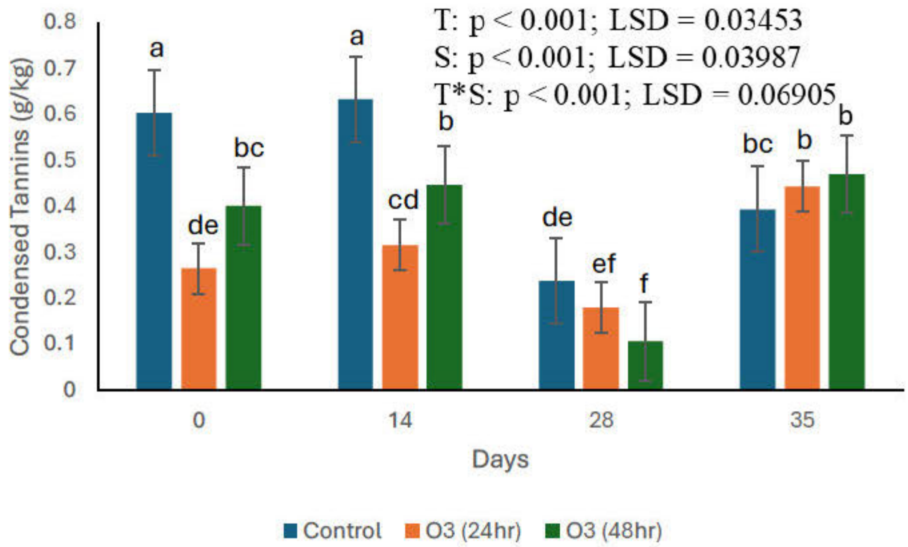


Figure 4.20: Effect of gaseous ozone on condensed tannins of 'Eureka' lemon peel during cold storage at 0°C for twenty-eight days and shelf-life at ambient temperature for seven days (±SE, n=5)

4.3.7 Principal Component analysis (PCA)

Table 4.1 Principal component analysis showing eigenvectors, eigenvalues and percentage variance of control, O3 (24h) and O3 (48h) in 6 parameters

Parameters	Factor scores (Treatments)		
	F1 (Control)	F2 (O_3 24h)	F3 (O_3 48h)
Condensed Tannins	-2.204	-0.021	0.008
DPPH	-0.364	0.003	-0.015
Flavonoids	1.045	-0.047	-0.062
Phenolics	2.564	-0.064	0.045
Ascorbic acid	1.024	0.141	0.003
Carotenoids	-2.064	-0.012	0.021
Eigenvalue	2.994	0.004	0.001
Variability (%)	99.815	0.148	0.037
Cumulative %	99.815	99.963	100.000

The PCA revealed 3 factor scores which accounted for 100% variation of total variation between the treatments (Table 4.1). Flavonoids, phenolics and ascorbic acid were strongly positively correlated to F1 and accounted for 99.815 %. This correlation is clearly visible on the Fig. 4.1, Fig. 4.2 and Fig. 4.5 as the highest concentrations were on the control. Conversely, DPPH and ascorbic acid were positively correlated to F2 and accounted for 0.148 % which was lower than F1. Lastly, condensed tannins, phenolics, ascorbic acid and carotenoids were positively correlated to F3, and accounted for 0.037 %. Ascorbic acid showed a positive correlation in all the three treatments indicating that as the treatment time increased, the ascorbic acid content also increased.

4.3.7.1 Principal component biplot analysis

Principal biplot analysis was used to visualize the relationship between the antioxidants and the treatments (Control, O_3 24h and O_3 48h). Parameters plotted close to the vectors showed strong correlation. For instance, phenolics, carotenoids and condensed tannins showed a positive correlation with the O_3 48h treatment. This indicates that the parameters were heavily affected by the treatment. As indicated in Table 4.1, vitamin C showed a positive correlation for all the treatments. This is visually clear on the Figure 4.7, as the ascorbic content is on the first quadrant, which is the positive quadrant. DPPH was correlated to the O_3 24h treatment, while flavonoids content was correlated to the control.

The present study revealed that different antioxidants thrive differently in different ozone concentration according to the oxidizing power of the ozone to them. The study revealed that phenolics, carotenoids, condensed tannins were affected by O_3 48h treatment, whereas DPPH and flavonoids were affected by O_3 24h and the control, respectively. Though the ascorbic acid accounted for positive correlation in all the treatment, it was however strongly affected by O_3 24h treatment. From these findings, the conclusion can be drawn that the antioxidants thrive differently in different ozone treatments, depending on exposure and the effect of the oxidizing power of the ozone.

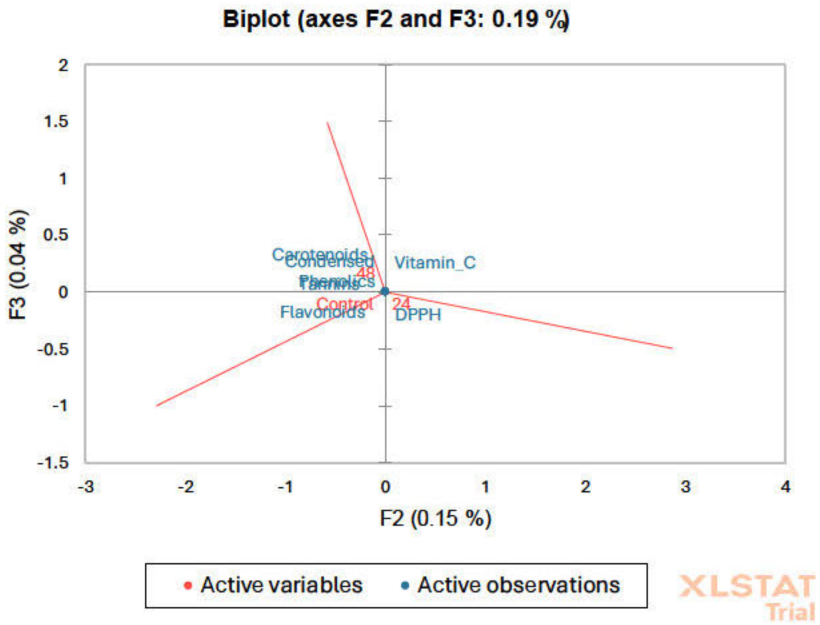


Figure 4.21: Principal component biplot loadings of F2 vs F3 showing the correlation of antioxidants of the 'Eureka' lemon exposed to 3 treatments (Control, O3 24h and O3 48) and stored at 0 °C, 95% RH and at ambient temperature for 7 days.

4.4 Conclusion

Gaseous ozone is a promising postharvest treatment for fresh horticultural produce. Our findings demonstrated that gaseous ozone is effective in preserving the quality of 'Eureka' lemon by minimizing depletion of various antioxidants. The influence of the treatments (O_3 24h and O_3 48h) varied among the antioxidants. The current results reveal that O_3(24h) had a positive influence on the phenolics, flavonoids and DPPH. However, O_3(48h) accounted for a positive effect on carotenoids, ascorbic acid and condensed tannins. These differences are attributed to the oxidizing power of the ozone. The current results provide a guide for an appropriate treatment exposure and storage days. Studies reveal that long-term ozone exposure could inhibit the levels of many antioxidants. However, our study revealed that short-term ozone exposure was successful in maintaining the antioxidants and biochemical attributes of the 'Eureka' lemon. Further investigation needs to be done on how the gaseous ozone influences the antioxidants of the 'Eureka' lemon when incorporated with other treatments such as edible coatings and modified atmosphere package (MAP).

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Chapter 5

The impact of gaseous ozone on senescence-related enzymes of 'Eureka' lemons during cold storage

Abstract

Enzymes are biological catalysts which play a critical role in fruit defence mechanisms against oxidative stresses. Postharvest treatments for prolonging the shelf-life of fresh produce can either enhance or inhibit the activities of the enzymes. This study assessed the impact of gaseous ozone on enzymes such as catalase, peroxidase, lipoxygenase and glutathione reductase as well as lipid peroxidation. 'Eureka' lemons were stored at 0°C, 95% RH for 28 days and thereafter kept at ambient temperature for 7 days. All the parameters were assessed over a 14-day interval. The spectrophotometric method was used in assessing the concentration of the targeted enzymes. The gaseous ozone significantly influenced ($p < 0.001$) most enzymes as there was an increase throughout the storage days (D_0 to D_{28}). For catalase, O_3(48h) accounted for a 72% increase, while for lipoxygenase O_3(24h) and control accounted for a 73% increase. The slight decline during ambient storage for catalase, lipoxygenase and glutathione reductase was observed. There is a need to further investigate the cause of a decline at ambient temperatures for the O_3(48h) treatment while the others were increasing as this has a negative impact on the production of the lemons.

Keywords: Citrus; 'Eureka' lemon, gaseous ozone, postharvest treatments, enzymes, quality, shelf-life.

5.1 Introduction

Citrus fruits are significant in human nutrition due to their health-promoting properties (Turner & Burri, 2013). They serve as a rich source of carbohydrates, organic acids, vitamins, carotenoids, flavonoids, and limonoids, which not only confer health benefits but also enhance aroma and flavor (Zhang et al., 2019; Baldwin, 1993). These compounds play a crucial role in determining fruit quality and shelf-life (White, 2002; Prasanna et al., 2007; Castellarin et al., 2011).

Lemons contain various enzymes that function as protective agents against oxidative stress, including catalase, peroxidase, lipoxygenase, and glutathione reductase. Enzymes associated with senescence in lemons are critical for maintaining fruit quality and longevity. For example, lipoxygenase (LOX) contributes to the breakdown of membrane lipids during cold storage, leading to chilling injury (Liao et al., 2022). The activity of LOX is linked to the formation of conjugated double bond systems in hydroperoxides (Liao et al., 2022).

LOX also plays a role in fatty acid metabolism within plants. In lemons, LOX is involved in synthesizing oxylipins, a class of bioactive compounds that bolster the plant's defense mechanisms against environmental stressors (Gomi et al., 2002). Sharma et al. (2016) demonstrated that elevated LOX activity in granulated fruits correlates with advanced senescence due to increased fatty acid oxygenation.

Catalase is another enzyme present in lemons, essential for converting hydrogen peroxide (H_2O_2) into water and oxygen. This enzyme acts as a vital antioxidant, safeguarding the fruit from oxidative stress associated with H_2O_2 accumulation during ripening (Olukanni et al., 2013). Catalase is predominantly located in the peel cells of lemons, which are rich in flavonoids (Wangab et al., 2020). The activity of catalase in fresh produce is influenced by factors such as oxygen concentration, temperature, and carbon dioxide levels (Ma et al., 2019).

Peroxidase is classified as an oxidoreductase and is known for catalyzing the oxidation of various organic and inorganic substrates using H_2O_2 as an electron acceptor (Twala et al., 2020). Among its multiple functions, peroxidase is involved in detoxifying harmful substances, regulating hormones, and generating reactive oxygen species (Twala et al., 2020). Additionally, it promotes lignification of cell walls, serving as a barrier against pathogen invasion and aiding tissue repair post-injury (Pandey et al., 2017).

Glutathione reductase (GR) is an enzyme that maintains cellular redox balance by controlling reduced glutathione levels within cells (Couto et al., 2016). GR plays a critical role in preserving the equilibrium between oxidized and reduced glutathione, thus aiding cellular defense against reactive oxygen species (ROS) and oxidative stress (Siboza et al., 2017). In lemons, GR facilitates the reduction of oxidized glutathione back to its active form, which is vital for protecting cells from ROS-induced damage. This enzyme is essential for maintaining cell membrane integrity and preventing lipid peroxidation during chilling stress (Siboza et al., 2017).

Research has explored the effects of postharvest treatments on citrus fruits. For instance, Kahramanoğlu et al. (2022) investigated hydrothermal treatments on ‘Newhall’ navel oranges and found that hot water dipping reduced respiration rates while enhancing the activity of specific enzymes such as polyphenol oxidase, peroxidase, and superoxide dismutase. Similarly, studies by Zhu et al. (2019) and Shah et al. (2019) examined the impact of gaseous ozone on 'Satsuma' mandarin and pummelo respectively; both studies reported increased antioxidant activity correlating with enhanced antioxidant enzyme activity. However, no research has specifically addressed the effects of gaseous ozone on senescence-related enzymes in 'Eureka' lemon. Therefore, this study aims to investigate the influence of gaseous ozone on selected enzymes in 'Eureka' lemon during cold storage.

5.2 Method and materials

5.2.1 Fruit material

Eureka' lemons were procured from Carisbrooke Citrus Valley Farm, Ixopo, KwaZulu-Natal (30.24172° S, 30.01315° E). Upon arrival at the Postharvest Laboratory of the Department of Horticultural Science, fruit were graded for uniformity according to colour and size, and thereafter stored in carton boxes. The experiment was arranged in a complete randomised design, each treatment had 3 replicates with each replicate containing 22 fruits.

5.2.2 Ozone treatment and cold storage

The fruit were treated with 0.3 ppm gaseous ozone equipped with an ozone analyzer (Corona discharge ozone generator, Ozone Purification Technology, Johannesburg, South Africa). Fruits were assigned to respective gaseous ozone treatments where time was the treatment factor: T1: Control (untreated fruit); T2: 0.3 ppm (24h); T3: 0.3 ppm (48h). After each ozone treatment the fruit was exposed to cold storage with 0°C with 95% RH for 28 days as per the shipping protocol for most export markets. The fruit were then exposed to ambient temperature (25 ℃) for 7 days.

5.2.3 Sample preparation

The sampling was done at 14-day intervals. On each sampling day (D_0, D_{14}, D_{28} and D_{35}), the portion of the flavedo (peel) was cut from the fruit and immediately stored at -22°C. The flavedo was thereafter freeze dried using Vir Tis BenchTop Pro freeze drier (SP Scientific, Warminster, PA, USA). Thereafter, the sample was pulverised into powder and kept at -20 ℃ until use. The sample extraction procedure varied from the parameters according to the method prescribed for each enzyme.

5.2.4 Determination of catalase

Catalase (CAT; EC 1.11.1.6) activity was assessed at 25°C following the methodology outlined by Kato and Shimizu (1987). The enzyme assay comprised 3.125 mM hydrogen peroxide (H_2O_2) in a 50 mM phosphate buffer at pH 7.0, combined with 200 µL of the enzyme extract, resulting in a total assay volume of 3 mL. Catalase activity was determined by measuring the decrease in absorbance of H_2O_2 at 240 nm, and the results were expressed as nanomoles of H_2O_2 decomposed per milligram of protein per minute (nmol H_2O_2 mg protein^{-1} min^{-1}).

5.2.5 Determination of peroxidase activity

Peroxidase (POD) enzyme extraction was performed following the protocol established by Criado et al. (2016), with slight modifications. In triplicate, 1 g of fruit sample was homogenized in 10 mL of sodium phosphate buffer (0.2 M, pH 7.0). The homogenate was then centrifuged at 15,000 rpm for 15 minutes at 4 °C using an Avanti J-265 XP centrifuge (Beckman Coulter, Indianapolis, USA). Subsequently, 0.1 mL of the enzyme extract was mixed with 2.7 mL of sodium phosphate buffer (0.05 M). Following this, 0.1 mL of hydrogen peroxide (1.5% w/v) and 0.2 mL of 1% p-phenylenediamine were added to the mixture. The absorbance was measured at 485 nm using a spectrophotometer (Shimadzu Scientific Instruments INC., Columbia, USA). Enzyme activity was quantified as the increase in absorbance at 485 nm per milligram of protein per minute.

5.2.6 Determination of Lipoxygenase (LOX)

Lipoxygenase (LOX; EC 1.13.11.12) was extracted using the method described by Rui et al. (2010), with minor modifications to ensure enzyme stability. Protein extraction was conducted at 4°C to minimize degradation. Flavedo powder (5 g dry weight) was combined with 5 mL of a 50 mM Tris-HCl buffer (pH 8), supplemented with 10 mM KCl, 500 mM sucrose, and 0.5 mM phenylmethylsulfonyl fluoride (PMSF), all prepared at 4°C. The mixture was homogenized and subsequently centrifuged at 12,000 × g for 10 minutes at 4°C. The supernatants obtained were utilized for the assessment of phospholipase D (PLD) and LOX activity.

LOX activity was quantified by preparing a standard assay mixture consisting of 200 µL Tween 20 and 40 µL linoleic acid in 40 mL of 0.1 mM phosphate buffer (pH 7.0). The reaction was initiated by adding 0.2 mL of the enzyme extract to 1 mL of the standard assay mixture. Absorbance was measured at 234 nm at room temperature (approximately 25°C). One unit of LOX activity was defined as the quantity of enzyme that produces an increase in absorbance at 234 nm of 0.01 min^{-1}. The protein concentration of the enzyme extract was determined using the Bradford assay (Bradford, 1976), and LOX activity was expressed as units per milligram of protein.

5.2.7 Determination of glutathione reductase (GR)

Glutathione reductase (GR) extraction was performed according to the method described by Sala and Lafuente (2000), with minor adaptations. Flavedo powder (1 g dry weight) was combined with 10 mL of a 100 mM potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA, and the mixture was maintained at 4°C to prevent enzyme degradation. The solution was then centrifuged at 27,000 × g for 15 minutes at 4°C, and the resulting supernatant was utilized for the assessment of GR activity as per the protocol established by Smith et al. (1988). One unit of GR activity was defined as the amount of enzyme that catalyzes the oxidation of 1 μmol of NADPH per minute. The protein concentration in the enzyme extract was determined using standard methods.

5.2.8 Determination of lipid peroxidation

The level of lipid peroxidation in lemon flavedo was quantified following the methodology described by Chong et al. (2005). A sample of lemon flavedo tissue (0.2 g dry weight) was homogenized in 4 mL of 0.1% (w/v) trichloroacetic acid (TCA) using a pre-chilled mortar and pestle at 4°C. The homogenate was then centrifuged at 3,000 × g for 5 minutes. Subsequently, 10 mL of the supernatant was transferred to a test tube containing a solution of 20% (w/v) TCA, 0.01% (w/v) butylated hydroxytoluene, and 0.65% (w/v) thiobarbituric acid (TBA). The samples were mixed vigorously and incubated at 95°C for 30 minutes before being cooled to 4°C and centrifuged again at 3,000 × g for 10 minutes. The absorbance of the resulting solution was measured at both 532 nm and 600 nm. Total malondialdehyde (MDA) equivalents were calculated and expressed as total MDA (nmol g^{-1} dry weight) according to the methods outlined by Siboza and Bertling (2013).

5.2.9 Statistical analysis

The collected data were analyzed using analysis of variance (ANOVA) with GenStat statistical software (GenStat 23rd Edition, VSN International Ltd, UK). Mean separation was conducted using Tukey's least significant difference at a significance level of 5%. Additionally, principal component analysis (PCA) was performed using XLSTAT 2024, allowing for the exploration of variable correlations and the identification of underlying patterns within the dataset.

5.3 Results and discussion

5.3.1 Catalase

Catalase (CAT; EC 1.11.1.6) is essential for protecting fruit against fungal infections by catalysing the decomposition of hydrogen peroxide into water and oxygen, thereby mitigating oxidative stress in fruits (Baker et al., 2023). In 'Eureka' lemons, ozone (O_3) treatment significantly ($p < 0.001$) influenced catalase activity (Fig. 5.1). However, the interaction between storage conditions and treatment did not have a statistically significant ($p > 0.005$) effect on catalase activity.

In this study, both the control and the O_3(24h) treatment exhibited a consistent increase in catalase (CAT) activity, although the O_3(24h) treatment resulted in higher concentrations. There is limited literature regarding the effects of gaseous ozone on catalase activity in citrus fruits, making this study one of the few to investigate this relationship. The findings align with those of Zhang et al. (2020), who reported similar results in strawberries.

The observed increase in catalase activity can be attributed to ozone's role in inhibiting antioxidants, thereby enhancing the plant's defense against oxidative damage (Li et al., 2022). Elevated catalase activity facilitates the scavenging of superoxide anions and hydrogen peroxide generated during storage (Zhang et al., 2020). Essentially, the potent oxidizing effect of gaseous ozone triggers the upregulation of CAT, which aids in neutralizing reactive oxygen species (ROS) and preserving fruit quality during storage.

In the O_3(48h) treatment, CAT concentrations showed a rising trend from Day 0 (D_0) to Day 28 (D_{28}). However, a sudden decline in CAT activity was observed during ambient storage. This decline may be linked to temperature fluctuations that affect the fruit's response to free radicals. Although ozone initially enhances CAT activity, prolonged exposure to gaseous ozone can lead to decreased activity (Piechowiak et al., 2020). Additionally, the decline in CAT activity may result from oxidative modifications to the enzyme itself due to excessive oxidative stress induced by ozone (Yaseen et al., 2014). The O_3(48h) treatment resulted in the highest increase in catalase activity from Day 0 to Day 35, with a 73% increase compared to control and O_3(24h) treatments, which showed increases of 69.36% and 71%, respectively.

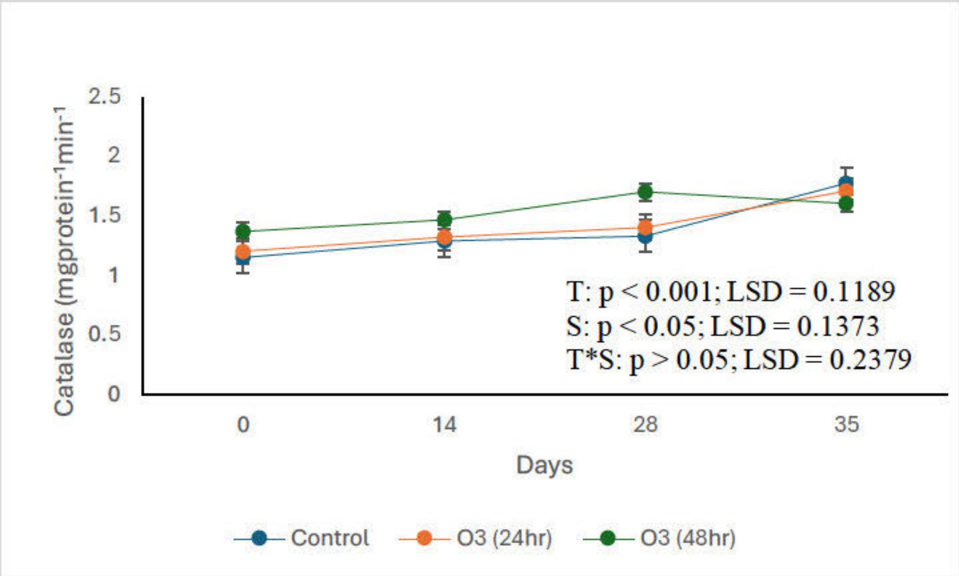

Figure 5.22: Effect of gaseous ozone on catalase concentration of 'Eureka' lemon peel during cold storage at 0°C for twenty-eight days and shelf-life at ambient temperature for seven days (±SE, n=3)

5.3.2 Peroxidase

Peroxidase in lemons is recognized as a critical component of the plant's defence mechanism against pathogens (Pandey et al., 2021). In this study, the interaction between treatment and storage did not significantly affect peroxidase activity ($p > 0.05$). However, both storage duration and treatment significantly influenced peroxidase activity in 'Eureka' lemons ($p < 0.001$).

The current findings demonstrated a consistent increase in peroxidase activity across all treatments (Control, O_3(24h), and O_3(48h)) (Fig. 5.2). These results are consistent with those reported by Zhu et al. (2019), who found that O_3 (2.5 μg L^{-1} for 24 h) enhanced peroxidase activity in 'Satsuma' mandarins over a 30-day storage period. The authors further noted that the increase in peroxidase activity could be associated with the presence of flavonoids and phenolics in fruits, which also contribute to the plant's defence mechanisms.

Ozone has the potential to activate the plant's antioxidant defence system and metabolize reactive oxygen species (ROS). The current results align with findings from Xu et al. (2019), which indicated that coriander exposed to 0.68 mg L^{-1} of ozone for 10 minutes exhibited an upward trend in peroxidase enzyme activity. Xu et al. (2019) argued that ozonation inhibits the accumulation of superoxide anions and hydrogen peroxide, which is linked to increased peroxidase activity.

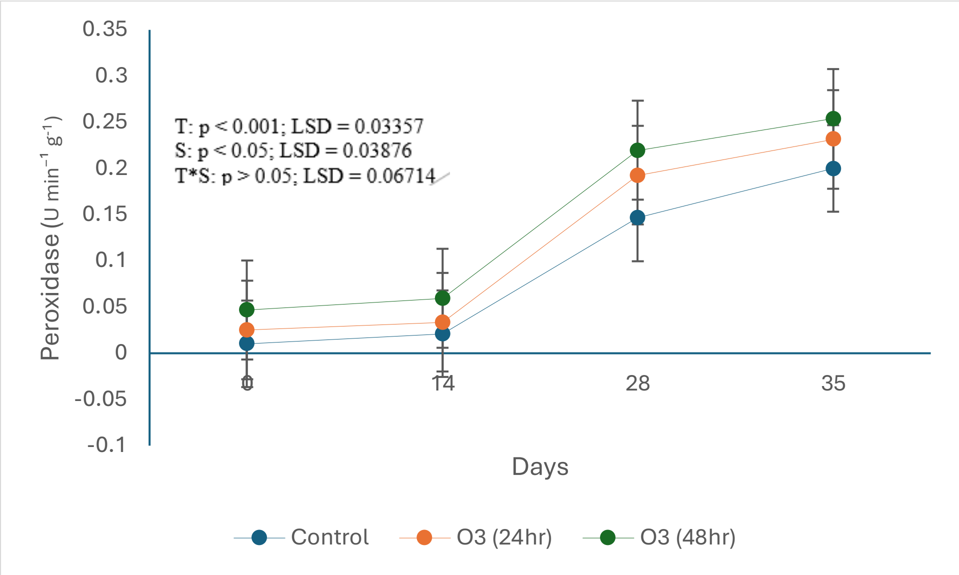

Figure 5.23: Effect of gaseous ozone on peroxidase of 'Eureka' lemon peel during cold storage at 0°C for twenty-eight days and shelf-life at ambient temperature for seven days (±SE, n=3)

5.3.3 Lipoxygenase

The interaction between treatment and storage conditions did not yield a statistically significant effect ($p > 0.05$) on the lipoxygenase levels in 'Eureka' lemons. However, both treatment type and storage duration had a significant impact ($p < 0.001$) on the concentration of lipoxygenase in the lemon peel.

The control and the ozone treatment for 24 h both exhibited a similar upward trend in lipoxygenase enzyme activity (Fig. 5.3). These results are consistent with findings by Modesti et al. (2018), which indicated that ozonated wine grapes (20 g h^{-1} over 3 days) experienced a more pronounced increase in lipoxygenase content compared to untreated samples and those treated with methyl jasmonate (MeJA) at concentrations of 10 µM and 100 µM. An elevation in lipoxygenase levels is beneficial as it aids in protecting the fruit from oxidative stress while facilitating the synthesis of important compounds such as arachidonic acid (AA) and linoleic acid (LA) (Modesti et al., 2018). These fatty acids are crucial for inflammatory responses and immune regulation. The heightened lipoxygenase concentration observed in ozonated fruit may be attributed to ozone's capacity to activate this enzyme (Wang et al., 2022). Lipoxygenase catalyzes the hydroperoxidation of polyunsaturated fatty acids, resulting in the production of hydrogen peroxide (H_2O_2), which can subsequently activate antioxidant enzymes within the fruit.

Conversely, while the ozone treatment for 48 h also resulted in increased lipoxygenase levels, a decline was noted between days 28 and 35. Ozone acts as a stress-inducing agent, leading to an accumulation of reactive oxygen species (ROS) during exposure. Thus, it can be posited that the prolonged ozone treatment may have adverse effects on specific biochemical parameters, particularly lipoxygenase activity. Mayookha et al. (2023) noted that ozone possesses the potential to inactivate certain enzymes, including lipoxygenase, by inducing oxidative stress that disrupts enzymatic pathways regulating lipoxygenase function. The elevated ROS levels are associated with the activation of antioxidant pathways, which may result in a decrease in lipoxygenase activity as a protective mechanism against oxidative damage (Modesti et al., 2021; Segade et al., 2017). The control and O_3(24h) treatments accounted for 73% of the observed effects, while O_3(48h) accounted for 66%.

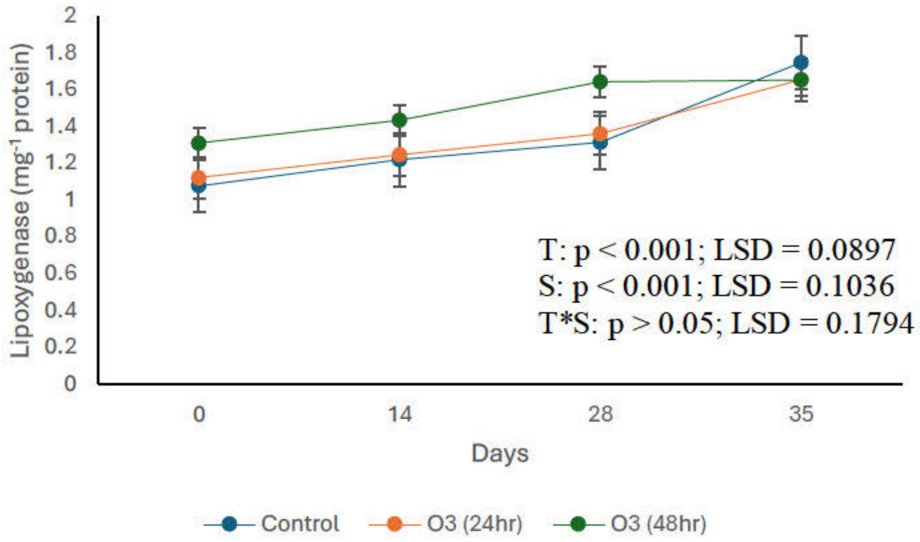

Figure 5.24: Effect of gaseous ozone on lipoxygenase of 'Eureka' lemon peel during cold storage at 0°C for twenty-eight days and shelf-life at ambient temperature for seven days (±SE, n=3)

5.3.4 Glutathione reductase

Glutathione reductase (GR) plays a crucial role in maintaining the equilibrium between oxidized and reduced glutathione, thereby contributing to cellular defense mechanisms against reactive oxygen species (ROS) and oxidative stress. In this study, GR levels were significantly influenced by the treatment ($P < 0.001$), while neither storage duration nor the interaction between treatment and storage had a statistically significant effect ($P > 0.05$) on GR enzyme activity.

All treatment groups (Control, O_3 for 24 h, and O_3 for 48 h) exhibited a consistent upward trend in GR levels throughout the storage period (Fig. 5.4). However, a slight decrease was observed in the O_3 for 48 h treatment on Day 35. These findings align with the research conducted by Piechowiak (2021), which investigated the effects of gaseous ozone on various fruits, including raspberry, 'Highbush' blueberry, strawberry, blackberry, and blackcurrant, at a concentration of 15 ppm for 30 minutes. Their study reported increased GR levels, which were attributed to enhanced mitochondrial function responsible for ATP production—the energy source essential for GR synthesis.

The observed decline in GR levels on Day 35 for the O_3(48h) can be ascribed to prolonged exposure to ozone and extended storage duration. While initial ozonation may enhance enzyme activities, extended storage often results in diminished enzymatic function due to accumulated oxidative damage and nutrient depletion (Zhang et al., 2020).

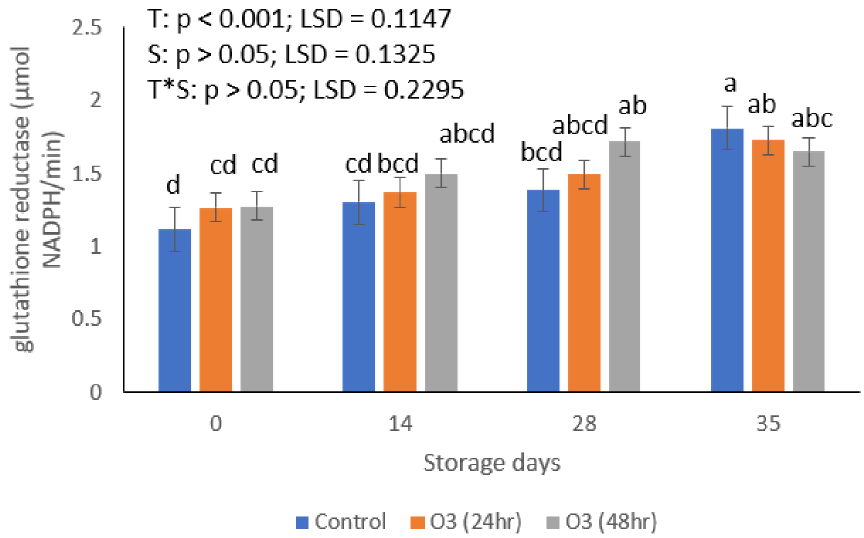

Figure 5.25: Effect of gaseous ozone on glutathione reductase of 'Eureka' lemon peel during cold storage at 0°C for twenty-eight days and shelf-life at ambient temperature for seven days (±SE, n=3)

5.3.5 Lipid peroxidation

Lipid peroxidation is a significant factor contributing to the degradation of food quality during storage and processing. This process involves the oxidation of unsaturated fatty acids within cellular membranes, resulting in considerable damage to fruit tissues (Lin et al., 2020). Lipid peroxidation leads to the generation of various oxidative byproducts, including hydrogen peroxide (H_2O_2), hydroxyl radicals, and reactive aldehydes, which can adversely affect proteins and contribute to overall deterioration (Geng et al., 2023). Statistically, lipid peroxidation levels remained unchanged across all storage days (D_0 to D_{35}) and treatments (Control, O_3 for 24 h, and O_3 for 48 h), with no significant differences observed ($p > 0.05$).

Exposure to gaseous ozone is known to induce lipid peroxidation due to the ozone's ability to trigger the formation of reactive oxidative species which can cause cell damage and deterioration in lipids. In their study on 'Clementina' mandarin, Iglesias et al. (2006) found that gaseous ozone increased the lipid peroxidation at the end of the 12-months storage to 98.3 nmol MDA g^{-1} FW. The current study showed varying findings. Our study showed that lipid peroxidation in treated fruit particularly on O_3(24h) in all storage days ranged between 4.95 nmol g^{-1} DW and 5.06 nmol g^{-1} DW. However, the O_3(48h) treatment showed an opposing trend. There was a constant trend of a decline in lipid peroxidation from D_0 to D_{35} as the MDA content decreased from 6.11 nmol g^{-1} DW to 4.98 nmol g^{-1} DW. This could be attributed to the activation of the antioxidants and enzymes after fruit exposure to gaseous ozone. Exposure to gaseous ozone is known to inhibit plant's defence system and help protect it against reactive oxidative species (ROS) (Hasanuzzaman et al., 2020).

5.3.6 Principal component analysis (PCA)

Table 5.1: Principal component analysis showing eigenvectors, eigenvalues and percentage variance of control, O_3 (24h) and O_3 (48h) in Catalase, Peroxidase, Lipoxygenase, Glutathione reductase and Lipid peroxidation.

Parameters	Factor scores (Treatments)		
	F1 (Control)	F2 (O_3 24h)	F3 (O_3 48h)
Catalase	-0.508	-0.028	0.003
Peroxidase	-1.774	0.066	-0.002
Lipoxygenase	-0.555	-0.025	0.019
Glutathione reductase	-0.486	-0.035	-0.020
Lipid Peroxidation	3.323	0.022	0.000
Eigenvalue	2.998	0.001	0.000
Variability (%)	99.946	0.049	0.005
Cumulative (%)	99.946	99.995	100.000

The PCA revealed 3 factor scores which accounted for 100% variation of total variation between the treatments (Table 5.1). For F1, it is only lipid peroxidation that showed a positive correlation and accounted for 99.946%. Peroxidase and lipid peroxidation showed a positive correlation on F2 and accounted for 0.049%. This is clearly shown on Fig 5.1 and Fig 5.3 which show lipid peroxidation and peroxidase, respectively. Catalase and lipoxygenase showed a positive correlation on F3 and accounted for 0.005%. Glutathione reductase showed a negative correlation in all 3 treatments, whereas lipid peroxidation showed positive correlation in 2 treatments (F1 and F2) and no correlation in F3.

5.3.6.1 Principal Component Biplot Analysis

Principal biplot analysis was used to visualize the relationship between the antioxidants and the treatments (Control, O_3 24h and O_3 48h). Parameters plotted close to the vectors showed strong correlation. This indicates that the parameters were heavily affected by the treatment. For instance, lipoxygenase and catalase are close to O_3(48). As indicated in Table 5.1, glutathione reductase shows a negative correlation to all the treatments. In Figure 5.5, GR is on the third quadrant which accounts for negative variables. Lipid peroxidation is positive in all the treatments and that is clearly shown in Figure 5.5. The enzymes behave differently to the treatments according to the oxidizing power of the ozone treatment and the ability of the enzyme to withstand it.

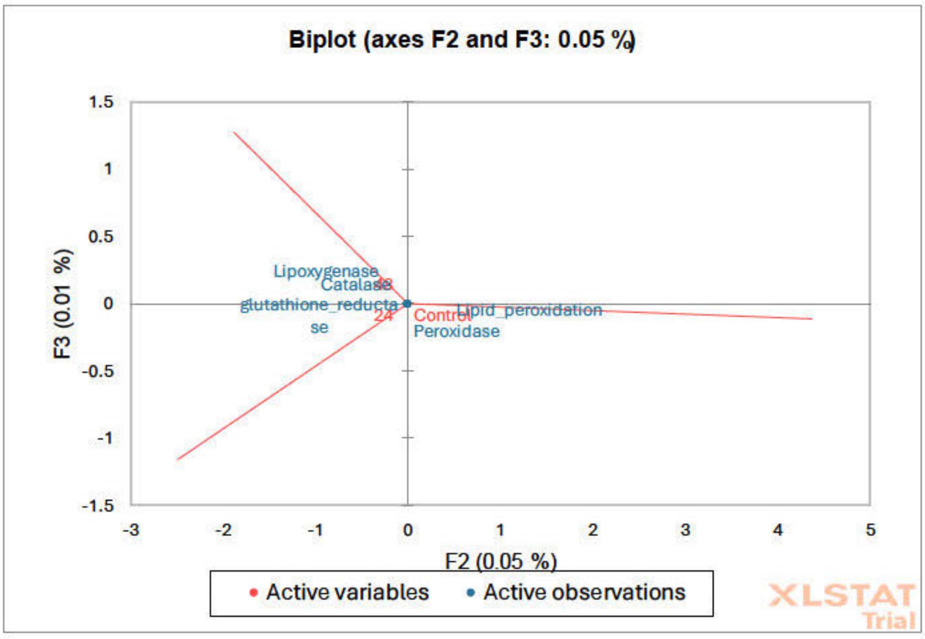

Figure 26.5: Principal component biplot loadings of F2 vs F3 showing the correlation of senescence-related enzymes of the 'Eureka' lemon exposed to 3 treatments (Control, O_3(24h) and O_3(48)) and stored at 0 °C, 95% RH for twenty-eight days and at ambient temperature for 7 days.

5.4 Conclusion

Gaseous ozone is a promising nonchemical treatment for preserving the quality of the 'Eureka' lemons. Our findings revealed that the gaseous ozone is able to minimise the risk of lipid peroxidation on the lemons which has implications for fruit quality and shelf-life. Furthermore, the study revealed that catalase, lipoxygenase and glutathione reductase were negatively affected by the O_3(48h) treatment towards the end of the storage period. This is a trend that needs more attention as the fruit might lose the nutritional quality and thereby decrease volumes sold by producers. On the other hand, all the treatments showed the positive influence on the peroxidase as there was an increase through all storage days. The study revealed that enzymes behave differently to the treatments depending on the oxidizing power of the ozone treatment. There is however a need to study more about what causes the decline in the three aforementioned enzymes particularly at the O_3(48h) treatment as various factors could be involved.

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Chapter 6

General discussion and conclusion

6.1. Introduction

Postharvest treatments are essential for reducing food loss by preserving the quality and extending the shelf life of fresh produce, including lemons. Lemons are classified as non-climacteric fruits due to their low ethylene production during maturation (Paul et al., 2011). They hold significant economic value, utilized across various industries such as food, pharmaceuticals, and cosmetics (Lorente et al., 2014; Arbona et al., 2015; Sánchez-Bravo et al., 2022). The quality of lemons is adversely affected by multiple factors, including handling, packaging, and storage conditions (Papoutsis et al., 2019; Kassim et al., 2020). These factors can lead to physiological and pathological disorders (Bhatta, 2022; Siboza et al., 2014), ultimately compromising the overall quality of the fruit (Mditshwa et al., 2017; Özdemir, 2021). Therefore, implementing postharvest treatments is crucial for maintaining quality and prolonging shelf-life.

This research study initially reviewed various postharvest treatments and their effects on the quality of citrus fruits. The review emphasized both conventional treatments and emerging technologies, such as gaseous ozone and low oxygen storage. Low oxygen environments have been shown to mitigate decay and enhance the overall quality of navel oranges (*Citrus sinensis* L. Osbeck, cv. Bellamy) (Archer et al., 2021), with a combination of low oxygen and low pressure demonstrating superior effectiveness. Furthermore, gaseous ozone was identified as a safe treatment that does not leave residues on fruit surfaces or pose risks to human health (Patil & Bourke, 2012). Gaseous ozone is particularly recognized for its effectiveness in pathogen elimination (Gartia-Martin et al., 2018), attributed to its antimicrobial properties (Bambalele et al., 2021). Additionally, gaseous ozone has been shown to enhance the antioxidant capacity of fruit and influence senescence-related enzymes (Zhao et al., 2013; Lin et al., 2019).

The first experiment aimed to evaluate the impact of gaseous ozone on the physical quality parameters of mature 'Eureka' lemons. The second experiment focused on assessing how gaseous ozone affects the antioxidant and biochemical properties of the fruit. Finally, the third experiment investigated the influence of gaseous ozone on senescence-related enzymes.

6.2. The effect of short-term gaseous ozone treatment on physical attributes

In this study, 'Eureka' lemons were treated with gaseous ozone at a concentration of 0.3 ppm for durations of 24 and 48 hours. Following the treatment, the fruit was stored under cold conditions at 0.0 °C and 95% relative humidity (RH) for 28 days, after which they were kept at room temperature for an additional 7 days. The results indicated a significant impact on both decay and firmness of the lemons, with the 48-h ozone treatment exhibiting a higher incidence of decay yet minimal loss in firmness compared to both the control group and the 24-h ozone treatment. This suggests that prolonged exposure to elevated ozone concentrations may compromise the integrity of cellular tissues and membranes, leading to increased fruit decay (Cao et al., 2022). Additionally, Panou et al. (2021) reported that fresh strawberries treated with ozone exhibited elevated pH levels, which can contribute to fruit decay (Ajayi & Olasehinde, 2009). This observation may help explain the lower decay incidence observed in untreated lemons.

The current study demonstrated that ozone treatment has the potential to reduce firmness loss in 'Eureka' lemons, with the 48-h ozone exposure resulting in comparatively lower firmness degradation. Bambalele et al. (2023) indicated that ozone can inhibit the activity of cell wall degradation enzymes, such as polygalacturonase and β-galactosidase. Mthembu et al. (2024) elaborated that enzymatic degradation and solubilization of cell wall polysaccharides (CWPs) lead to structural and compositional alterations in the cell wall, which contribute to textural changes and subsequent fruit softening.

The control group exhibited greater mass loss compared to the ozonated fruits. Specifically, by the end of the storage period, the control group experienced a 16% mass loss, which was higher than the 10% and 9% losses observed in the O_3(24h) and O_3(48h) treatments, respectively. Li et al. (2022) argued that ozone treatment activates antioxidant defence mechanisms that help metabolize reactive oxygen species (ROS). This activation results in an increase in enzymes such as superoxide dismutase and peroxidase, which mitigate ROS and preserve cellular integrity during storage. Furthermore, ozone reacts rapidly with ethylene, inhibiting its production and thereby limiting mass loss (Liu et al., 2021). Consequently, the O_3(48h) treatment was effective in minimizing mass loss.

Colour is a critical parameter influencing consumer preference and is often used as an indicator of fruit maturity. From Day 0 to Day 35, the control group showed a colour improvement of 43%, while O_3(24h) exhibited a 52% improvement, and O_3(48h) demonstrated the greatest improvement at 54%. This suggests that O_3(48h) effectively preserved fruit colour. Ozone has been shown to enhance antioxidant levels in fruits, including phenolic compounds and carotenoids (Li et al., 2022), which are vital for lemon fruit coloration. The oxidative stress induced by ozone can stimulate the production of secondary metabolites that significantly contribute to fruit pigmentation (Li et al., 2022).

6.3. The effect of gaseous ozone in antioxidant and biochemical attributes

This chapter examined the effects of gaseous ozone on the antioxidant and biochemical properties of 'Eureka' lemons. The treatment significantly influenced carotenoids, ascorbic acid, and condensed tannins ($p < 0.001$), while no significant effects were observed for phenolics and flavonoids ($p > 0.05$). The O_3(24h) treatment positively impacted the levels of phenolics, flavonoids, and DPPH radical scavenging activity. However, a decline in flavonoid content was noted from Day 0 to Day 35; despite this, the O_3(24h) treatment exhibited a lesser decline compared to other treatments. This observation may be attributed to ozone's ability to enhance the antioxidant capacity of fresh produce, thereby delaying senescence.

Conversely, the O_3(48h) treatment resulted in significant increases in carotenoids, ascorbic acid, and condensed tannins ($p < 0.001$) from Day 0 to Day 35, with the O_3(48h) treatment showing the most substantial increase. Carotenoids are responsible for the yellow pigmentation of the fruit (Lana et al., 2020), and these findings align with colour changes observed in Chapter 3, where the O_3(48h) treatment demonstrated higher colour values. The activation of antioxidative systems during ozone exposure likely contributes to the elevated ascorbic acid content observed in the O_3(48h) treatment compared to others. Ascorbic acid plays a critical role in helping fruit withstand oxidative stress during ozonation (Miller et al., 2021; Liu et al., 2021). The increase in condensed tannins is noteworthy, as this study represents one of the few investigations into their influence in fruits, revealing that O_3(48h) effectively enhanced condensed tannin levels in 'Eureka' lemons.

6.4 The impact of gaseous ozone on senescence-related enzymes

The impact of gaseous ozone on 'Eureka' lemons was meticulously investigated in this study. The results indicated that all enzymes, with the exception of lipid peroxidation, were significantly affected by ozone treatment ($p < 0.001$). Notably, peroxidase activity exhibited a consistent upward trend from Day 0 to Day 35. Xu et al. (2019) noted that ozonation inhibits the accumulation of O_2 and H_2O_2, which is associated with the increase in peroxidase activity.

However, the O_3(48h) treatment negatively influenced enzyme levels by the end of the storage period. This abrupt decline in enzyme activity may be attributed to storage temperature effects on their response to free radicals. Piechowiak et al. (2020) reported that prolonged exposure to gaseous ozone can decrease the activity of certain enzymes, including catalase. Increased catalase activity is beneficial as it aids in scavenging superoxide anions and hydrogen peroxide generated during storage (Zhang et al., 2020), which may explain the observed increase in peroxidase activity. The activation of antioxidant pathways is correlated with elevated reactive oxygen species (ROS) levels, leading to the degradation of specific enzymes such as lipoxygenase and glutathione reductase (Modesti et al., 2021).

Lipid peroxidation levels varied across treatments; specifically, O_3(24h) resulted in increased lipid peroxidation, whereas O_3(48h) led to a decrease. Gaseous ozone is known to induce lipid peroxidation due to its capacity to trigger the formation of reactive oxidative species, which can damage cellular structures and lead to lipid deterioration (Iglesias et al., 2006).

6.5. Conclusion and future prospects

This study carefully assessed the influence of the gaseous ozone on the overall quality and shelf-life of the ‘Eureka’ lemon. This study is the first to provide an understanding of mode of action for ozone application in ‘Eureka’ lemon. From this study, it was demonstrated that ozone treatment duration is very critical as the fruit quality attributes behaved differently among the treatments. It was revealed that for most attributes or parameters, higher ozone duration are likely to adversely affect fruit quality. For instance, decay and most of the enzymes were severely affected by the O_3(48h) treatment. It is therefore safe to conclude that O_3(48h) or increasing the ozone duration is less likely to be recommended as it possesses higher oxidizing power which negatively impacts fruit quality. On the other hand, fruits under O_3(24h) did not show any signs of severe deterioration and most biochemical attributes and antioxidant enzymes responded positively under the treatment. Future studies should investigate performance of O_3(36h), which is slightly lower than the 48h which carries a higher potential to adversely affect the fruit. The combination of the gaseous ozone and edible coatings should also be studied as the edible coatings are known to provide a protective layer against postharvest losses including water loss. It also eliminates most of the pathogens that are likely to develop during storage. More research is needed to elucidate the mechanisms by which gaseous ozone affects microbial load and fruit physiology at a molecular level. Understanding these mechanisms could lead to more targeted applications and improved treatment strategies.

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