Extension of Avocado Fruit Postharvest Quality Using Non-Chemical Treatments

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Abstract: Developing postharvest management techniques using environmentally friendly and non-chemical approaches is key to extending the shelf life of avocados in a safer and health conscious manner. Avocados are prone to postharvest deterioration caused by mechanical damage, chilling injury, soft landing, uneven ripening and decay. Among the different cultivars of avocados commercially grown worldwide, the ‘Hass’ variety continues to be the most predominant due to its nutty flavour and functional properties. Most of the literature on postharvest decay and disorders affecting avocado fruit quality during storage and marketing is dedicated to the Hass avocado. Some of these postharvest problems are unique to the ‘Hass’ avocado can possibly be controlled by simply investing more research into other cultivars. These postharvest losses can be significantly controlled using eco-friendly technologies, such as modified atmosphere, physical heat treatments and most importantly investing in natural biodegradable products with naturally inherent antimicrobial properties. Thus, this review includes the recent research-based information on the use of non-chemical treatments on the improvement of fruit health and quality.

Keywords: avocado; shelf life; induced defence mechanism; phenolic compounds; antimicrobial activity

1. Introduction

The avocado (Persea americana Mill.) is an important sub-tropical climacteric fruit characterised by a green peel and a creamy, buttery pulp. Fresh avocado oil (~11%–19%) is comprised of monounsaturated fatty acids (MUFA ~71%), saturated fatty acids (SFA ~16%) and polyunsaturated fatty acids (PUFA ~13%) [1]. These oils regulate bad cholesterol (Low density lipoprotein, LDL) levels in blood with good cholesterol (High density lipoprotein, HDL), and improve the bioavailability of fat-soluble vitamins and phytochemicals [1]. Avocados are a good source of folic acid necessary for developing the foetal neural tube. They provide antioxidants (lutein and zeaxanthin) essential for the brain and the prevention of age-related macular degeneration, cataracts, and neurodegenerative diseases like Alzheimer’s [2,3].

The potassium derived from avocados helps in regulating blood pressure, a key factor for the prevention of heart attacks, strokes and kidney failure [3]. They are also a good source of fibre and minerals, such as copper, phosphorus, magnesium, manganese, iron and zinc. Extracts of avocado oil are used for cosmetic facial and body lotions because of their immense regenerative and soothing properties. In the culinary space, avocados are used in making salads, sandwiches, savoury dips, smoothies and a variety of other dishes.

The global trading of fresh avocados generates immense revenues, especially from export proceeds. In 2018, approximately USD $5.6 billion was generated from 6.4 million tons of avocado global produce and of this, 2.4 million tons went to exports [4]. In the same year South Africa exported 57,665 tons
earning USD $71.93 million (DAFF, 2017). By country, Mexico was the leading export nation accounting for approximately 43%, followed by Netherlands (13.2%), Peru (13%), Spain (6.2%), Chile (5.8%), USA (3.2%), Kenya (2.1%), South Africa (2.1%), New Zealand (1.3%) and Colombia (1.1%). The largest European avocado consumer markets are, Netherlands, France, Germany, Spain and the UK, which import their avocado supplies from South Africa, Israel, Chile and Colombia.

The upward trend in avocado consumption of ready-to-eat and trading is driven by consumer purchasing power [5]. Main drivers for avocado consumption are ripeness, appearance, taste, quality, nutrition, convenience and value for money [6]. Avocados ripen only after harvest unlike other climacteric fruits and ripens takes place in ambient conditions for five to six days after harvesting and most consumers do not have the patience to wait out this process and may be turned off from buying the fruit [7]. To encourage continuous avocado consumption and improve consumer accessibility, retailers have introduced two types of marketing campaign ‘ripe and ready’ to eat fruit on the shelf or ripe at home within five days. The ‘ripe and ready’ to eat fruit has set a new standard of convenience and consumers are prepared to pay premium prices, 30% higher, vs. ripe at home within five days fruit [7]. To ensure a year-round supply of avocados, European supermarkets source a wide variety of produce with good external and internal qualities from South Africa, Israel, Chile and Columbia. The physical attributes green skin and black or purple skin avocados include commercial fruit weight and size, shape (oval or pyriform), degree of uniform skin colouration, skin texture (smooth or rough), absence of defects (e.g., sunburn) and internal flavour, seed size, flesh texture and pulp colour.

There are over a hundred commercial cultivars of avocados grown globally and these can be classified into Guatemalan, Mexican, West Indian and hybrids [8]. Popular commercial South African cultivars include Guatemalan ‘Hass’ and ‘Reed’, hybrids ‘Fuerte’, ‘Ettinger’, ‘Pinkerton’ and Ryan’ and ‘Zutano’ (Mexican) [8]. Most of the cultivars share similar features, with slight differences in size, colour, flavour, shape and peak growing season. The ‘Hass’ is the predominant variety grown worldwide and is associated with a buttery, nutty flavour, and spherical shape that turns from bold green to dark purplish-black on ripening [9,10]. ‘Hass’ fruit and its sub-cultivars, such as ‘Lavi Hass’, ‘Lamb Hass’ and ‘Gem Hass’ have an extended shelf-life and give better economic revenue when compared to other cultivars in the world [1]. The ‘Hass’ variety makes up South Africa’s largest percentage (40%) of avocado exports and comprises more than 80% of avocado production in the USA and Australia [1,11]. Despite its commercial popularity, the marketing of ‘Hass’ is constrained by several postharvest disorders. Ideally, ‘Hass’ fruit’s colour should turn purple/black from green during ripening; however, the uniform black or purple colour development in this cultivar is often affected during ripening. As a result, huge postharvest losses are incurred from rejected fruit for not meeting the consumers’ expectation with regards to fruit quality standards. It is possible that some of the problems predominantly unique to ‘Hass’ can be controlled by regulating the mechanisms involved in the biosynthetic pathways of anthocyanin development in avocado pericarp (skin) during ripening in avocado cultivar Hass. Furthermore, due to the global popularity of the avocado ‘Hass’ cultivar, it takes centre stage in terms of production volumes due to higher consumer acceptance, market sales and research and development. Therefore, this review has been dedicated to this variety alone across the globe.

The typical commercial supply chain of avocados starts from the orchard, then to the pack-house cold store before heading to local retail and export markets. Avocados can be transported either by truck, railroad, aircraft and ship depending on the destination and travel time. The atmospheric conditions in these transport modes can vary between regular atmosphere (RA), refrigerated temperatures <10 °C, and controlled atmosphere (using 2%–5% O2 and 3%–10% CO2). Throughout the supply chain, the fruit temperature and surrounding atmosphere must be monitored in order to prevent fruit ripening and decay. Road shipments usually utilise refrigeration to delay transit ripening of fruit [12]. The RA conditions are not really designed for long distance transport and marketing and rather more applicable for short distance transportation over short time intervals preferably using airfreight prior to cold storage. For domestic supply chain the fruit is packed in consumer pack with modified packaging to
maintain quality during marketing. Controlled atmosphere (CA) and 1-MCP technology are employed when the fruit must be kept in prolonged storage especially when destined for overseas markets [13]. With CA storage, the shelf life of firm avocados can extend up to 6 weeks. The CA environmental conditions range around 5–12 °C; 95% RH using 2%–5% O₂ and 3%–10% CO₂ depending on the cultivar [13,14]. Modern CA containers can be electronically programmed to create active/intelligent atmospheres that can monitor and appropriately modify the storage atmosphere and temperature end route. Furthermore, the CA chambers can be manipulated to change from a storage condition into ripening rooms by altering atmospheric temperature and gas composition [13]. Therefore, this review summarises the causes of postharvest quality constraints of avocado and prevention methods safe and environmentally friendly non-chemical strategies based on the published research publications.

2. Constraints during Marketing

The postharvest quality of avocados in general is limited by mechanical damage, soft landing, uneven ripening, chilling injury (CI), grey pulp, vascular browning and insects. The prevalence of these constraints facilitates loss in cosmetic appeal and the development of fruit decay. To extend the shelf life of avocados, fungicide applications are predominantly applied to arrest fruit decay development. Fungicides typically kill fungi by disrupting the cell membranes, deactivating critical enzymes processes, and disturbing key energy production including respiration [15]. However, because the mode of action of these fungicides is so specific, any small changes in the target fungi’s genetic makeup can compromise their efficiency resulting in some populations developing resistance to future applications [16]. Chemical fungicides are potentially carcinogenic and have been reported to negatively interfere with the normal functioning of the female reproductive system [15]. Furthermore, traceable chemical residues can remain on the surface of plants, which can be potentially toxic and pose a health risk for consumers. The disposal of these fungicides is also a problem as they are associated with long degradation periods and build-up, leading to environmental pollution.

Consumer awareness regarding the drawbacks associated with fungicide applications have necessitated the enforcement by importing countries for strict regulations regarding the maximum residue limits (MRL) permissible in the edible portion of the fruit [16]. Consequently, several research efforts aimed towards finding safer alternative technologies have been made in the past decade or so. These alternative methods can be classified into physical heat treatments (such as hot water dips, steaming, dry heat and forced air), irradiation, controlled atmospheres, electrolysed water, biological control, plant extracts (such as essential oils) and elicitors (e.g., chitosan and jasmonates) [17]. This review explores the different types of postharvest constraints of avocados and the non-chemical treatments available to alleviate their impacts.

2.1. Mechanical Damage

Mechanical injuries can occur at point from harvest and packing throughout to retail and these can be in the form of lenticel damage and abrasions on the peel surfaces [18]. Most of the injuries can act as port of entries for postharvest pathogens after ripening during marketing. Therefore, severely damaged fruits can be easily isolated and culled before marketing [17]. Injuries, such as lenticel damage, instantly become apparent after any form of mishandling as they cause small dark spots about 1–5 mm diameter appear, which are more severe when fruit are harvested wet [19]. Poor physical handling and squeezing of individual fruit during selection by consumers also contributes to mechanical defects [19]. Dropping green avocado fruit from as little as 0.1 m is enough to induce bruising and loss of cosmetic appeal [16]. Visible symptoms of mechanical injury (scrapes, bruises, abrasions) in avocado fruit reveal the presence of cellular and tissue damage. The cellular disruption facilitates polyphenoloxidase (PPO) activity to accelerate browning of the bruised mesocarp tissue [19].

Flesh (mesocarp) bruising is an important barrier to purchasing—more so than price [20]. At least 80% of fruit on retail display can have some degree of internal quality issues, mainly flesh bruising [20]. Fruit bruising provides an infection pathway, increasing the incidence of brown patches [21]. Using
appropriate packaging to cushion fruit from mechanical injuries can significantly help alleviate the problem. Identifying practices that contribute to mechanical bruising and preventing defect fruit from reaching the retail shelf is key to reducing fruit losses and ensuring consumer confidence. A new suspended fruit packaging system was recently developed to ensure damage-free transport of soft fruit [22]. The authors demonstrated that soft fruit could be effectively cushioned from transit vibration damage using the suspended tray packaging system [22].

Near infrared spectroscopy (NIRS) can be used to assess fruit quality along the supply chain [23]. This non-invasive technology measures the internal/external quality attributes of horticultural produce via the near infrared part of the light spectrum to determine chemical composition. Fourier transform near-infrared reflectance (FT-NIRS) in diffuse reflectance mode was successfully used to predict the quality attributes of whole ‘Hass’ fruit [23]. The authors correctly classified >90% of the population according to maturity and principal eating quality attributes based on dry matter (%DM); the presence of bruises [23]. They could also predict the ‘export potential’ of avocados based on the risk of developing external and internal defects as an indication of potential shelf life [23]. In a similar study, the use of proton magnetic resonance imaging (1H-MRI) was used to non-destructively monitor bruise expression over time of ‘Hass’ avocados [10]. The 1H-MRI technique clearly identified fruit morphological features and bruised mesocarp tissue. The advantages of non-destructive technologies are they are applicable to all kinds of biological materials, the cost of analysis is low, and sample preparation is simple without any chemical reagent needed. In addition, the technology can run simultaneous analysis of multiple constituents, with good repeatability and high throughput capability. However, non-destructive technology has its drawbacks. For instance, the process of calibration development is a major impediment as the reference sample collection and processing can be a time-consuming and economically expensive exercise based on the type of analysis [23].

2.2. Soft Landing and Uneven Ripening

Quality and logistical problems influence avocado market prices and limits traders’ power to negotiate for better profit margins. Export avocados have ripening difficulties and are prone to fruit softening especially during long storage intervals [24]. Soft landings occur when the fruit ripen prematurely during storage and arrive soft in overseas markets [25]. Soft arrivals of fruit cause immediate marketing constraints, since shelf life of softer fruit is very limited. Fruit with undesirable colouration often remain inedible and rubbery, with poor flavour development [11,26]. The situation is exacerbated by the fact that additional labour costs will be necessary to sort and select fruit of acceptable firmness [27]. Diseased fruit are completely unacceptable; it is usually fruit that ripen before reaching their market destination that are likely to develop fungal infections than fruit that arrives in a hard condition. ‘Hass’ avocados in particular take longer to ripen, and are prone to uneven colouration during ripening [25]. The problem of ripening heterogeneity of “Hass” avocados limits the volumes of uniform quality fruit reaching the trigger ripe and ready to eat markets [27].

To address the problem of uneven colouration, the storage and ripening conditions need to be controlled. Throughout the ripening process, the humidity (90%–95%), carbon dioxide (<1%), pulp temperature (15 to 20 °C), firmness and airflow must be constantly monitored. High (>30 °C) temperatures can cause irregular ripening, flesh darkening, development of off-flavour and decay [9,24]. Too low (<10 °C) temperatures can suppress ripening process and induce chilling injuries. For South Africa, managing soft landing and uneven ripening issues ensures the supply of excellent quality hard export avocados fruit for marketing late in the season when the demand is big and the profit at its highest [25]. There are various measures that can be taken to control ripening disorders of avocados and some of these are summarised in Table 1.

2.2.1. 1-Methylcyclopropene (1-MCP)

Avocado soft landing can be controlled by using a plant growth regulator called 1-methylcyclopropene (1-MCP; SmartFreshSM) [14], as demonstrated in Table 1. The 1-MCP primarily
prevents subsequent ‘soft landings’ from occurring by reducing the risk of ripening taking place during transport [28]. The treatment delays rate of respiration and weight loss, and increases the antioxidant content during fruit storage [28]. It also reduces the prevalence of ethylene associated physiological mesocarp discoulouration (grey pulp) and internal chilling injury. In addition, 1-MCP can also influence the reduction of fungal infections that can occur as a result of premature softening during transport [14]. The effect of harvest time and 1-MCP (300 ppm) was evaluated on the ‘Hass’ avocado fruit skin colour during ripening. Treated, fruit were stored at 5.5 °C for 28 d, followed by ripening at 21 °C [11]. The treatment improved the colour development for early and middle harvest when compared with late harvest [11]. The authors concluded that, 1-MCP had the potential to alleviate the colour change problem through an extension of the ripening time for the early and middle harvest season. The potential for 1-MCP to delay ripening in green skin cultivar ‘Pollock’ avocado fruit placed under tropical ambient storage conditions was investigated [29]. The 1-MCP treatment doubled the fruit shelf life from 4 d up to 9 d, when compared against the controls. A significant (p < 0.05) delayed change in fruit peel colour, glossiness and softening was recorded in the 1-MCP treated fruit compared to the controls. 1-MCP treatment effectively maintained the antifungal activity, and minimised the undesirable ripening-related changes that were observed in fruit kept under warmer ambient conditions [29].

Table 1. Measures to control ripening disorders of avocados.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Treatment Conditions</th>
<th>Outcome</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Hass’</td>
<td>Heat shock (38 °C) Nitrogen shock (1 kPa O₂ and 1 kPa CO₂) CA: 4 kPa O₂ and 6 kPa CO₂ for 21 d</td>
<td>Treatment significantly reduced ripening heterogeneity in early and middle season fruit, while N₂ + CA did not</td>
<td>[7]</td>
</tr>
<tr>
<td>‘Hass’</td>
<td>1-methylcyclopropene (1-MCP; 300 ppm) Storage: 5.5 °C for 28 d, and ripened at 21 °C</td>
<td>Improved colour development for early and middle harvest by over 50%</td>
<td>[11]</td>
</tr>
<tr>
<td>‘Hass’</td>
<td>1-MCP; SmartFreshSM Storage 30 d at 5 °C prior to ripening in ambient conditions</td>
<td>Prevented subsequent ‘soft landings’ Delayed rate of respiration and weight loss Reduced risk of inter-fruit ripening variability Reduced incidence of mesocarp discoulouration</td>
<td>[15]</td>
</tr>
<tr>
<td>‘Pollock’</td>
<td>1-MCP; 300 nL/L Stored at 27 ± 2 °C and 65 ± 2% RH</td>
<td>Shelf life extended by 2.2 fold from 4 d to 9 d Controlled Stem end rot, delayed changes in peel colour, fruit softening and glossiness</td>
<td>[30]</td>
</tr>
<tr>
<td>‘Hass’</td>
<td>Hot water treatment (38 °C) + controlled atmosphere (CA: 4 kPa O₂ and 6 kPa CO₂) stored for 30 d at 5 °C</td>
<td>Reduced the glycolytic throughput Induced protein degradation to deliver energy for alternative ripening pathways, and accumulation of soluble sugars (sucrose and galactinol)</td>
<td>[31]</td>
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</table>

2.2.2. Heat Treatments

Ripening heterogeneity of ‘Hass’ fruit can be effectively alleviated by using heat treatment [23]. A hot water treatment (38 °C) used in conjunction with a controlled atmosphere (CA: 4 kPa O₂ and 6 kPa CO₂) prior to storage for 30 d at 5 °C induced the development of soluble sugars (sucrose and galactinol) and stress-related enzymes [28]. Integration of metabolomics and proteomics data revealed a positive correlation between reduced glycolytic throughput and induced protein degradation with the
heat shock treatment. L-isoleucine, L-valine, L-aspartic and ubiquitin carboxyl-terminal hydrolase were used to correlate for protein degradation between treatments [27]. One research group investigated the effect of two postharvest pre-treatments (nitrogen shock N₂ and heat shock) prior to controlled atmosphere (CA) on controlling ripening heterogeneity of ‘Hass’ avocado [7]. The results demonstrated how heat shock prior to CA storage could significantly suppress uneven ripening in early and middle season fruit, while N₂ + CA did not. Additionally, none of the treatments altered the fatty acid profile. Their results pointed to ripening synchronisation in ‘Hass’ avocado being related to induction of metabolic processes related to ethylene (biosynthesis), and the efficiency of the heat treatment [7].

2.3. Chilling Injury (CI) and Pulp Spot

Low temperature storage (5–13 °C) is the first measure taken to extend the postharvest quality of avocados. Refrigeration maintains quality preservation by suppressing the speed of cell metabolism and plant senescence development [30]. However, exposing avocados to temperatures below their critical threshold (10–15 °C), but above freezing, can permanently cause irreversible damage to plant tissues, cells and organs leading to pulp spot and chilling injury [31]. ‘Pulp spot,’ a low-temperature disorder, is commonly described as small dark spots in the flesh, and blackening of a region surrounding cut vascular bundles, which are either immediately visible when the fruit is cut, or which develop within a few minutes after cutting. Both pulp spot and CI disorders involve browning reactions implicating particularly the enzyme polyphenol oxidase (PPO).

The extent to which the avocado can be chilled depends on the cultivar [31]. In ‘Hass’ avocado fruit, chilling injury (CI) symptoms tend to occur after at least 4 weeks of storage at about 6 °C, depending on maturity and growing conditions. External symptoms of avocado CI are skin pitting, scalding, water-soaked appearance, failure to ripen, blackening, off-flavour and decay [9,32]. Internally, CI symptoms can be evidenced by flesh browning (grey pulp, pulp spot and vascular browning), and increased susceptibility to pathogen attack [9]. Symptoms of this physiological disorder become more evident when produce are transferred to room temperature [33].

Chilling injury of avocados can be controlled by applying physiological heat shock treatments (Table 2). Short term high temperatures (>35 °C) induce protective plant defence stress responses and help repair damage to membranes, organelles, or metabolic pathways [32]. In addition to, alleviating CI symptoms, postharvest heat treatments can be applied for insect disinfestation, disease control and modifying fruit responses to cold [34]. In one study, CI of ‘Hass’ fruit was effectively suppressed using dry (50% RH) and moist (95% RH) forced air at 38 °C for 6 h before storage (5 °C, 85% RH up to 8 weeks) [35]. Fruit heated with dry air exhibited the best internal quality and the lowest CI incidence [35]. However, the heat treatments induced higher weight loss and respiration rate (Table 2). The integration of postharvest heat treatments into the commercial postharvest chains should therefore be integrated with caution. Although they may reduce CI and control decay, they can also seriously damage fruit sensory quality, causing deterioration of flavour and aroma, and therefore, consumer rejection [28]. In a similar study, ‘Hass’ avocados were kept in CA (O₂—2%; CO₂—2%) at 5 and 7 °C to see if chilling injury could be prevented at the higher temperature [14]. Both CA storage regimes, at 5 °C or 7 °C, resulted in better fruit quality than for control fruit kept in normal air at 5 °C. However, after 4 weeks of storage, 7 °C was less effective at retarding the progression of ripening in storage than CA at 5 °C as shown in Table 2.

Exogenous polyamines, such as methyl jasmonate, can be used to control CI by simply enhancing plant defence mechanisms against the disorder [28]. These polyamines can induce synthesis of certain stress proteins (heat shock and pathogenesis-related proteins), maintain lipid oxidation stability and extend the shelf life of avocado fruit kept under low temperature storage [36]. Dipping ‘Hass’ and ‘Ettinger’ avocado fruit in 2.5 μM and 10 Mm MJ methyl jasmonate solution for 30 s inhibited the development of CI in fruit that had been subsequently stored for 2 weeks at 1 °C [37]. It was further confirmed that methyl jasmonate and methyl salicylate compounds can maintain the postharvest quality of cold stored ‘Hass’ avocado fruit by altering their fatty acid content and composition [38].
The fruit were kept at 2 °C for 21 days followed by 6–7 days shelf-life at 20 °C, simulating supply chain conditions, and experienced reduced CI especially at 100 µmol/L [36].

### Table 2. Measures to control chilling disorders of avocados.

<table>
<thead>
<tr>
<th>Cultivar</th>
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<th>Outcome</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Hass’</td>
<td>Dry (50% RH) and moist (95% RH) forced air at 38 °C for 6 h Storage (5 °C, 85% RH up to 8 weeks)</td>
<td>Dry air exhibited the best internal quality and the lowest CI - Heating induced higher weight loss and respiration rate</td>
<td>[35]</td>
</tr>
<tr>
<td>‘Hass’</td>
<td>CA (2% O₂ and 2% CO₂ at 5 and 7 °C)</td>
<td>Better keeping quality under CA conditions CA at 7 °C was less effective as 5 °C at controlling ripening leading to decay</td>
<td>[32]</td>
</tr>
<tr>
<td>‘Becon’</td>
<td>1-MCP (1 µL/L) and stored in low oxygen Stored at 4 °C for 21 d in 3.5% O₂, followed by 20 °C for 14 d</td>
<td>Severity of CI was totally impaired Reduced fruit softening and cell membrane permeability Storage life extended by 15 d more</td>
<td>[34]</td>
</tr>
<tr>
<td>‘Hass’ and ‘Ettinger’</td>
<td>Methyl jasmonate (MeJA; 2.5 µM and 10 Mm) 1-MCP; 300 ppb for 18 h Low-temperature conditioning (LTC; a gradual decrease in temperature over 3 days)</td>
<td>Combined treatment inhibited CI development in fruit stored for 2 weeks at 1 °C by more than 2 fold Longer shelf life and less decay at 1 °C compared to 5 °C</td>
<td>[37]</td>
</tr>
<tr>
<td>‘Hass’</td>
<td>MeJA or MeSAvapour at 10 and 100 µmol/L Low temperature storage: 2 °C for 21 d followed by 6–7 days shelf-life at 20 °C</td>
<td>CI reduced by 50%–60%, best at 100 µmol/L Sifted membrane integrity and fatty acid content Down regulated LOX gene expression and lipoxygenase activity</td>
<td>[38]</td>
</tr>
<tr>
<td>‘Hass’</td>
<td>CA (10 kPa CO₂ + 5 kPa O₂ at 5 and 20 °C)</td>
<td>Reduced ethylene production and senescence Shelf life extended by 2 d based on firmness Early CA maintained firmness but induced discoloration 7 d after removal from CA</td>
<td>[39]</td>
</tr>
<tr>
<td>‘Hass’</td>
<td>Pre-conditioning (pulp hydro-cooling to 6 °C) and waxing Fruit kept at ambient storage at 3 °C for 46 d</td>
<td>Three-fold reduction of internal damage Retarded fruit colour break and controlled rate of fruit metabolism and ethylene production</td>
<td>[40]</td>
</tr>
</tbody>
</table>

The LOX gene expression and lipoxygenase activity was also down-regulated [36]. In a related study, the severity of CI was totally alleviated using 1-MCP (1 µL/L) and low oxygen (3.5% O₂) [36]. The effect of CA (10 kPa CO₂ + 5 kPa O₂ at 5 and 20 °C) was investigated in avocados at different intervals for early, middle or late stages of postharvest storage [39]. Irrespective of timing, respiration rate of avocado stored at 20 °C was reduced while under CA environment (Table 2). The early stage (postharvest storage) fruits exposed to CA, retained fruit firmness better however, it revealed a high incidence of internal discolouration after 7 days that means after the samples were withdrawn from the CA storage. At 5 °C, the avocado skin colour and flesh discolouration were maintained better by CA treatment compared to control fruit [39]. The CA reduced endogenous ethylene production, resulting in lighter, more vivid and firmer fruit, suggesting a reduction in senescence. The commercial application of early CA in avocado packaging suggests that shelf life could be extended by 2 d based on firmness. Cold damage during storage of ‘Hass’ avocado can also be controlled through pre-conditioning (pulp hydro-cooling to 6 °C) and waxing [40]. The hydrocooling pre-treatment alone cannot effectively prevent lenticelosis, but in combination with waxing creates a three-fold reduction
of internal damage and retarded fruit colour break [40]. The treatment-controlled rate of metabolism and ethylene production extended the commercialisation period of the fruit kept at room temperature after storage at 3 °C for 46 days [40].

2.4. Fruit Health and Diseases

Avocado fruit health is predominantly limited by postharvest anthracnose and stem-end rot caused by Colletotrichum gloeosporioides (Penz.) Penz. & Sacc., and Lasiodiplodia theobromae, (Pat.) Griffon & Maubl respectively [41]. These fungal diseases account for perhaps 70% of all fruit losses. Most fungal infections are initiated in the orchard and their impact are often seen during postharvest causing severe produce losses [42]. The basic principles of disease management involve prevention (providing protection) and therapy (treatment or cure) [43]. Fungi often spoil stored fruit through development of body rots causing blemishes to edible parts and reducing appearance and market value. Effective disease management strives on anticipating occurrence of disease and attacking vulnerable points in the disease cycle (i.e., weak links in the infection chain). Postharvest diseases of avocado are commercially controlled by the application of fungicides such as prochloraz; they control infections already established in the surface tissues of produce and protect against infections, which may occur during storage and handling. To avoid over reliance on fungicides, various alternative treatments can be exploited to extend avocado fruit health postharvest. These treatments include elicitors, plant extracts and environmentally friendly biodegradable polymers.

2.4.1. Activation of Signal Molecules in Plant Defences

Plants possess inherent biochemical and structural defence systems, which defend them from infection. Some of these mechanisms are already in place before pathogen invasion (i.e., constitutive resistance) and others only become active in response to infection (i.e., induced resistance). Unripe fruit are concentrated with preformed antifungal diene compounds that are fungitoxic. The decline to sub-lethal concentrations of these compounds are triggered by fruit ripening, which facilitates pathogen development and growth [44]. The concentrated prevalence of constitutive antifungal compounds in the unripe peel of avocado fruit significantly contributes to the quiescence of C. gloeosporioides [29]. As corroboration, the germination and growth of latent C. gloeosporioides was positively correlated with the decline of persin and epicatechin compounds [41]. Improving the individual potential of the host to respond to pathogen attack through activating defence mechanisms at the biochemical and molecular level offers a viable disease control strategy [44,45]. Resistance can be induced locally in the attacked tissue or spread via signalling through the plant or even to neighbouring plants, resulting in systemic acquired resistance (SAR).

Levels of the diene compounds can be enhanced by applying various treatments, including physical treatments (e.g., heat, ultrasound, radiation) and elicitors (e.g., salicylic acid, phosphonates, jasmonates, salicylic acid and chitosan). Postharvest thermal treatments can trigger the biosynthesis of heat shock proteins (HSP) in the fruit tissue protecting cellular components. Heat treatments work by either destroying the pathogen and its propagules, or simply disrupting cellular growth and development processes following treatment [7]. Heat can be applied in the form of either hot water or hot air; hot water is a more efficient medium for heat transfer than hot air.

2.4.2. Plant Defence Elicitors

Plant elicitors represent resistance inducers that can prompt gene expression of pathogenesis related (PR) proteins, synthesis of phytoalexins, and reactive oxygen species (ROS) to protect the plant during stress [46]. The application of thymol as a fumigant significantly retained the severity and prevalence of artificially and naturally inoculated anthracnose avocado cvs. Hass and Ryan [46]. Thymol fumigation triggered a higher activity of both β-1,3-glucanase and chitinase in treated avocado fruit compared to the commercial prochloraz treatment. The β-1,3-glucanases represents PR-2 proteins that are responsible for catalysing the endotype hydrolytic cleavage of 1,3-β-D glycosidic linkages in β-1,3-glucans present
in the cell wall of many pathogenic fungi. Chitinases, on the other hand, catalyse the cleavage of the bond between C1 and C4 of two consecutive N-acetyl-d-glucosamine monomers of chitin, a common component of the fungal cell wall and exoskeleton of arthropods.

**Silicon**

Postharvest application of Silicon (Si) offers beneficial protective properties to plants. This was evidenced by the increase in total phenol concentration, lipid peroxidation as well as polyphenol oxidase and catalase activity in ‘Hass’ treated fruit [47]. The treatments involved soaking the fruit in potassium silicate (0, 5, 13 or 25 × 10³ mg/L) for 25 min prior to cold storage (5.5 °C) for 17 days, followed by five days at room temperature (25–30 °C). The silicon treatment applications had no effect on respiration rate but positively influenced retention of fruit firmness, weight loss, and mesocarp electrical conductivity (EC) [47]. As an elicitor, silicon improved the accumulation of free polyphenol concentrations. These phenolics are operational in the induction/repression of genes as well as the activation/deactivation of enzymes involved in key metabolic pathways. The presence of these phenolic compounds is important towards enhancing the fruit’s ability to endure stressful conditions better in order to maintain overall quality attributes for extended periods of time.

**Jasmonates**

Jasmonates are lipid-derived compounds that act as vital signalling compounds that facilitate plant stress responses and developmental processes. As signal molecules, salicylic acid (SA), jasmonic acid (JA), and methyl jasmonate (MeJA) endogenous plant growth substances can significantly influence development and responses to environmental stresses. Jasmonates are known to control many responses to cell damage and invasion of pathogens in plant and also play a role in reproduction [37]. From literature, it can be perceived that the acquired systemic resistance related to the signalling mediated by MeJA is linked to important signal transduction systems [37]. The introduction of MeJA to the plant activates catalytic enzyme activities involved in biosynthetic reactions to form defence compounds such as polyphenols, alkaloids, reactive oxygen species (ROS•), or PR proteins [37]. Resistance can be induced in diseased using biotic and abiotic elicitors as a strategy to contain spread of fungal infection. When applied to ‘Hass’ avocado fruit at 10 and 100 mmol/L, these plant signalling compounds effectively stimulated natural defences against both biotic and abiotic stress [45]. Anthracnose disease incidence was significantly reduced in ‘Hass’ fruit treated using MeJA or MeSA vapours, especially at 100 mmol/L. [45]. The treatment enhanced the activity of chitinase, β-1,3-glucanase PAL, and epicatechin. This highlights that exposure to MeJA or MeSA vapours prior to cold storage offers a potential alternative strategy for disease control to commercial fungicide application.

2.4.3. Essential Oils (EOs)

Essential oils can have the potential substitute/complement fungicides and therefore much research effort has been dedicated towards understating their properties and application benefits as demonstrated in Table 3. These plant extracts comprise of a mixture of different terpenoid compounds and their oxygenated derivatives. They can be applied on fruits directly (through vapor phase, spraying, dipping and fumigation) or indirectly as part of film and coating matrices. As antifungals, their mechanism is to generate instability of biomolecules, such as DNA, lipids, and proteins, which are vital for the correct cellular functioning of pathogens [46,48]. The hydrophobic fractions of EOs can dissolve in the hydrophobic components of the pathogen’s cell membrane thereby disrupting membrane integrity and permeability to Adenosine-5’-triphosphate (ATP) and ultimately causing cell death [48]. EOs can also interfere with enzymatic reactions, leading to improper arrangement of cell wall components, chitin, glucans and glycoproteins and cell leakage of Ca²⁺, Mg²⁺ and K⁺. As elicitors, treated fruit can experience an increase in antioxidant levels (polyphenols, flavoids, anthocyanins) and oxygen absorbance capacity. The treatments can also enhance the resistance of plant tissues against
pathogens and reduce physiological deterioration. Plant resistance is achieved through the induction of defence compounds (chitinase, \( \beta \)-1,3-glucanase and peroxidise) [44,46].

Essential Oils as Fumigants

A major advantage of EOs is their bioactivity in vapour phase, a phenomenon that makes them ideal as applicable fumigants for the preservation of stored products. A variety of EOs have presented numerous beneficial properties towards extending the shelf life of avocado fruit postharvest. Thyme EO and its components thymol and carvacrol for example, have all been presented to possess important antifungal activity in their vapour state [49]. For this reason, much research on different ways to apply thyme oil has been published over the last decade [46]. Several other EOs have also demonstrated positive effects on avocado fruit. The fumigation of ‘Fuerte’ avocado with citral significantly reduced the incidence of stem-end rot by 75% and induced the activity of active defence-related enzymes in the fruit [50]. The application of savory and thyme oils at 2000 ppm presented strong antifungal activity, and significantly reduced expansion of necrotic lesions around the inoculation sites on avocado fruit by 58%–64% [51]. Even the fruit firmness assay showed that savory and thyme oils treatments had a positive influence on the fruit textural attributes as the treated fruit were 2.5–3.3 times firmer than the controls [51].

Lemongrass essential oil was used in various formulations for the control of anthracnose in ‘Fuerte’, ‘Hass’ and ‘Ryan’ avocado fruit [6] as shown in Table 3. The treatments definitely suppressed the prevalence of anthracnose, gray pulp, vascular browning, weight loss, loss of fruit firmness and showed acceptable taste, flavour, texture and higher overall acceptance of naturally infected fruit [6]. Similarly, the fumigation of ‘Fuerte’ avocados with thymol also demonstrated an effective control of anthracnose (\( C. \) gloeosporioides) in a separate study [48]. In both case studies, the enzyme activity of polyphenol oxidase (PPO), which is responsible for grey pulp disorder in avocado fruit, was significantly higher in the untreated control fruit, but low and non-significant between the treatments [48].

The advantage of applying thymol fumigation is the elicitation of important defence response at the enzymatic and transcript level making the fruit less susceptible to fungal development post infection (Table 3). As biological fumigants, essential oils elicit the incidence of enzyme activities and genes expressed in fruit after storage and they have demonstrated to have to phytotoxic effect of the pericarp of the fruit when applied as in vapour form [50]. Studies have shown that major constituents of savory oil and thyme oil, which are carvacrol (71.2%) and thymol (73.3%), portray a greater potential as natural fungicides through complete inhibition of the mycelia growth [52]. The predominating volatile compound (thymol) of thyme oil significantly inhibited the growth of \( C. \) gloeosporioides and \( L. \) theobromae in avocado fruit at the concentration of 66.7 \( \mu \)L/L after storage [53]. In a related study, savory oil at an EC\(_{50}\) of 1507.19 \( \mu \)L/L effectively reduced the incidence of anthracnose on avocado fruit by 64.07% [52].

Thyme oil vapour was shown to induce pathogenesis-related proteins, phenylalanine ammonia lyase and lipooxygenase genes in ‘Hass’ and ‘Ryan’ avocado fruit and concurrently retained the fruit quality [44]. The exposure to thyme oil vapours induces the expression of pathogenesis-related (PR) genes, i.e., chitinase and \( \beta \)-1,3-glucanase in ‘Hass’ and ‘Ryan’ avocados [46].

These enzymes are capable of hydrolysing polymers of fungal cell walls and are therefore implicated in the plant defence mechanisms, reducing the fruit decay [44]. The PAL gene is a key factor in the phenylpropanoid pathway and is involved in the biosynthesis of phenolic compounds, such as phenolic acids and flavonoids. Epicatechin compounds enhance the antioxidant capacity and ROS scavenging activity, thereby enhancing resistance to pathogen invasion [47]. Epicatechin can be linked to lowering the reduction rate of antifungal diene compounds through delaying the expression of the LOX genes. Therefore, the down regulation of LOX gene expression in ‘Hass’ and ‘Ryan’ avocado can be ascribed to be the prime mechanism by which thyme oil vapours contribute to enhanced plant resistance (Table 3). Thyme oil vapours probably induce the fruit to activate their defence mechanisms and provide protection against anthracnose causing pathogen (\( C. \) gloeosporioides). At the same time,
another part of the thyme oil’s mode of action involves morphological alterations of the hyphae and spore viability [46].

Table 3. Alleviation of postharvest diseases of avocados using essential oils-based emulsions.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Treatment Conditions</th>
<th>Outcome</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Hass’ and ‘Ryan’</td>
<td>Thymol (96 µL/L) vapour</td>
<td>Elicited activities of β-1,3-glucanase and chitinase; Higher enzyme activities observed in ‘Ryan’ than ‘Hass’</td>
<td>[46]</td>
</tr>
<tr>
<td>‘Hass’</td>
<td>KSi (0, 5, 13 or 25 × 10³ mg/L) prior to storage (5.5 °C; 17 d), and 5 d at 25 °C</td>
<td>Increased total phenols, lipid peroxidation, catalase activity; Retained fruit firmness, weight loss, and shelf life</td>
<td>[47]</td>
</tr>
<tr>
<td>‘Hass’</td>
<td>MeJA and MeSA at 10 and 100 mmol/L,</td>
<td>Reduced anthracnose incidence by ~40%; Enhanced chitinase, β-1,3-glucanase, PAL, and epicatechin</td>
<td>[45]</td>
</tr>
<tr>
<td>‘Fuerte’</td>
<td>Citral Essentil oil (EO); Thymol drench solutions (0.1% v/v)</td>
<td>23%–48% reduction in stem-end rot; Induced the activity of defence-related enzymes</td>
<td>[48]</td>
</tr>
<tr>
<td>‘Booth 7’</td>
<td>Savory and thyme EO</td>
<td>Reduced expansion of necrotic lesions by 58–64%; Treated fruit were 2.5-3.3 times firmer</td>
<td>[51]</td>
</tr>
<tr>
<td>‘Fuerte’</td>
<td>Savory EO at an EC₅₀ of 1507.19 µL/L</td>
<td>64.07% inhibition of Anthracnose in vivo</td>
<td>[53]</td>
</tr>
<tr>
<td>‘Hass’</td>
<td>Chitosan-pepper tree essential oil biocomposite</td>
<td>Controlled C. gloeosporioides; Prevented quality deterioration for 10 d</td>
<td>[43]</td>
</tr>
<tr>
<td>‘Hass’</td>
<td>55% chitosan nanoparticles (CSNPs) + thyme (3 and 5%) EO, 8 d</td>
<td>100% control of C. gloeosporioides in vitro and 60% in vivo</td>
<td>[49]</td>
</tr>
<tr>
<td>‘Fuerte’ and ‘Hass’</td>
<td>Chitosan (0.5–1.0% w/v) fused with a moringa extract (2%)</td>
<td>Improved fruit quality; Mannoheptulose maintained by eight-fold</td>
<td>[54]</td>
</tr>
<tr>
<td>Hass’ and ‘Gem’</td>
<td>Carboxyl methylcellulose (CMC 1% w/v) + moringa leaf and seed extracts (2%)</td>
<td>Impaired pathogen growth; Improved fruit shelf life</td>
<td>[55]</td>
</tr>
<tr>
<td>‘Fuerte’</td>
<td>Pectin-based coating fruit kept at (5, 10 and 20 °C)</td>
<td>Extended the shelf life to over a month especially at 10 °C; Suppressed CO₂ evolution, weight loss and colour change</td>
<td>[56]</td>
</tr>
</tbody>
</table>

Essential Oils as Drench Solutions

Essential oils can be integrated into drench solutions, which are operable in packhouses on bulk fruits for short periods. Although some of the solutions can have phytotoxic effect on the fruit surface, this can be averted by dissolving the essential oils in emulsifiers to give a uniform mixture (Table 3). Thymol drench solutions (0.1% v/v) were effectively used in a commercial packhouse setup to control the incidence of anthracnose and induce the activity of phenylalanine ammonia lyase, chitinase and β-1, 3 glucanase in fruit inoculated with L. theobromae and C. gloeosporioides [52]. Essential oils can also be incorporated with other compounds, including fungicides to improve fruit quality and reduce postharvest decay. This effect was demonstrated through the combination of prochloraz® (500 µg mL⁻¹; P50) with 0.1% v/v thyme oil [52]. A significant reduction in stem-end rot incidence of 10% was observed, while P50 (500 µg mL⁻¹) + 0.1% v/v thyme oil induced the activities of phenylalanine ammonia lyase, chitinase and β-1, 3 glucanases in avocado fruit inoculated with L. theobromae and C. gloeosporioides [50].
Table 4. Examples for antifungal waxes, film matrices and inserts for active packaging of avocados.

<table>
<thead>
<tr>
<th>Active Compound</th>
<th>Packaging Material</th>
<th>Antifungal Activity</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µL Lemogass EO</td>
<td>Whatman filter paper strip (3 cm²) impregnated with EO in tea bags</td>
<td>Significantly reduced grey pulp, vascular browning, weight loss and incidence of C. gloeosporioides by 50%</td>
<td>[6]</td>
</tr>
<tr>
<td>10–20 wt% thymol</td>
<td>Polylactic acid (PLA)-based polymer</td>
<td>Controlled C. gloeosporioides</td>
<td>[57]</td>
</tr>
<tr>
<td>5 wt% thyme EO</td>
<td>A trilayer low-density polyethylene (LDPE) film</td>
<td>Controlled C. gloeosporioides</td>
<td>[58]</td>
</tr>
<tr>
<td>Thyme EO 5 or 10 wt%</td>
<td>Pulverized polyethylene impregnated sachets</td>
<td>Facilitated retention of dietary phytochemicals, such as p-coumaric, ferulic and caffeic acid, catechin and epicatechin, fatty acids, D-mannoheptulose sugar</td>
<td>[58]</td>
</tr>
<tr>
<td>Carnauba wax containing Lippia scaberrima EO</td>
<td>Wax coating</td>
<td>Control of anthracnose</td>
<td>[59]</td>
</tr>
<tr>
<td>Candelilla wax and antioxidant ellagic acid (0.01 wt%)</td>
<td>Wax coating</td>
<td>Controlled growth and spread of anthracnose, preserved the appearance and overall fruit quality during 6 weeks of storage</td>
<td>[60]</td>
</tr>
</tbody>
</table>

Incorporation of Essential Oils into Packaging Films

The volatility of EOs limits their application as food preservatives as they are thermally unstable and may require incorporation into suitable matrices to achieve prolonged release [56]. This can be addressed by incorporating EOs into edible films and coatings for practical application as active packaging to extend the shelf life of a wide variety of foods, including avocados (Table 4). Incorporating essential oils with edible coatings, acceptable to the organic market, offers additional protection while maintaining the overall fruit quality, nutritional compounds and consumer acceptance of the produce [16]. Such application methods are more beneficial than simple spray applications. Incorporating EOs directly into coating matrices effectively reduces the inoculum severity of the postharvest pathogens present on the fruit surface. These active matrices can simultaneously prevent dehydration, microbial growth, oxidative rancidity, surface browning and oil diffusion. The prevalence of C. gloeosporioides was effectively inhibited in avocados kept at 25 °C using a polylactic acid (PLA)-based polymer incorporated with 10%–20% thymol [58]. In a similar study, thyme oil was integrated into a trilayer low-density polyethylene (LDPE) film as an antifungal active additive for avocado packaging (Table 4). The films exhibited great in vivo antifungal activity using 5wt % thyme oil against C. gloeosporioides [58]. Furthermore, the incorporation of thyme oil did not change the water vapour transmission characteristics of the original film [58]. In addition to the antifungal activity, impregnation of thyme oil into low-density polyethylene impregnated pellets (TO-LDPE-P) facilitated retention of dietary phenolics such as p-coumaric, ferulic and caffeic acid, catechin and epicatechin, fatty acids, and also by maintaining the threshold of D-mannoheptulose sugar enabled the retention of fruit quality in ready-to-eat avocado [58].

Integration of Essential Oils with Nanotechnology

Nanomaterials provide greater advantages such as bioavailability, controlled release, protection and better compound performances compared to micrometric materials. Biodegradable nanoparticles loaded with essential oils can be used to reduce water losses on coated fruit and control fungal decay by damaging the spore membrane integrity, thereby creating a homeostatic disequilibrium and cell death [43]. In this view, a chitosan-pepper tree essential oil biocomposite was synthesised by nano-precipitation
technique and its anti-fungal activities were evaluated on ‘Hass’ fruit previously inoculated with *C. gloeosporioides* [43]. After 10 days of storage there was significant preventive and curative activity against *C. gloeosporioides*, with no visible internal damage or loss of other quality parameters, such as water loss, and firmness changes were also positively regulated [43]. The synergistic effect of EO can be utilised to enhance the antimicrobial potential of biodegradable antimicrobial biopolymers. Incorporation of thyme (3% and 5%) essential oil to a nanostructured edible coating based on 55% chitosan nanoparticles (CSNPs) improved the control of *C. gloeosporioides* and this was demonstrated through a complete growth inhibition of the pathogen [49]. There was a notable reduction in the incidence of *C. gloeosporioides* on avocado cv. Hass by up to 60% [49]. Also, at the end of the 8-day storage period, the nano-coating had no adverse effects on the quality of avocado; moreover, fruit firmness was better maintained than untreated fruit [51].

### 2.4.4. Biodegradable Polymers

Avocado fruit health can be conserved by simply packaging or coating with eco-friendly biodegradable polymers that are preferably of an edible nature. This approach is important in addressing problems associated with waste disposal, toxic residues and a large environmental footprint [60]. Biodegradable coatings maintain fruit quality by decreasing the desiccation occurring during storage [60]. They regulate water vapour, oxygen and carbon dioxide transfer in or out of the produce, thereby influencing the ongoing respiratory activity and produce quality [56]. When used properly, biodegradable polymers can delay fruit ripening, suppress decomposition of chlorophyll, reduce the weight loss, retain the ascorbic acid, improve the appearance of the fruit, and prolong the shelf life. These polymers can be derived from plant extracts and shells from crustaceans. Below are some examples of biodegradable polymers that have been applied on avocado fruit postharvest.

**Chitosan**

Chitosan is a natural, biodegradable, non-toxic, bioactive polymer with fungicidal effects. It is synthesised by a number of living organisms including crustaceans and commonly used to carry volatile compounds [61]. The polymer is made up of polycationic characteristics [poly (β-(1-4)-N-acetyl-d-glucosamine)] produced through the N-deacetylated derivative of chitin [61]. The interaction of this biopolymer with pathogenic microorganisms disrupts normal cell permeability affecting biochemical processes, such as homeostasis, fungal respiration as well as nutrient uptake and the synthesis of proteins leading to severe cell damage [62]. To the plant host, chitosan induces plant defence systems, by stimulating the production of important enzymes (phenylalanine ammonium lyase, polyphenol oxidase, etc.) and plant immunity, favouring the adaptation of plants to biotic and abiotic stresses. In addition, it has the capability to form a mechanical barrier (coating) which helps control respiration rate, prevent water losses, maintain fruit firmness and colour, and extend shelf life.

Edible coatings derived from chitosan exhibit film-forming properties that create a modified atmosphere around the product which improves its shelf life [61]. Weight loss is prevented by the chitosan’s barrier properties, which control water vapour transfer between the fruit and the environment. Firmness retention can be associated with modifications of gas concentrations in fruits which regulate enzyme activities of polygalacturonase and pectin-esterase, associated with pectin depolymerization and softening of fruit [61]. Treated fruit also shows a lower ethylene production and respiration rate when compared to uncoated fruit [63].

Chitosan formulations can be merged with a variety of antimicrobial compounds to extend produce shelf life. The combination of chitosan (1.5%) with 2% propolis inhibited the mycelia growth of *C. gloeosporioides* from avocado in vitro and significantly reduced the incidence and severity of anthracnose in the fruit in vivo [60]. Postharvest treatment of ‘Fuerte’ and ‘Hass’ avocado fruit chitosan (0.5%–1.0% w/v) fused with a moringa extract (2%) significantly improved fruit quality of both cultivars and the mannoheptulose was maintained eight-fold [63]. Chitosan activates differential expressions of genes and metabolic changes that ensue as an effect of precise recognition of progression between
the host and pathogen [61]. The gene expression for treated fruit can be comparably different to uncoated infected [64,65]. Studies indicate that chitosan application triggers persin biosynthesis, and this in turn protects of avocado fruits against C. gloeosporioides [65]. Chitosan at 1.5% considerably reduced the incidence of stem-end rot and anthracnose in both inoculated and naturally infected avocados [64]. The chitosan coating induced the defence-related genes through the up-regulation of PAL and down-regulation of LOX genes, which moderately allowed higher epicatechin contents in the exocarp, which could have contributed to improved anthracnose control [66]. Furthermore, chitosan solution (1.5%) retained moderate levels of C7 sugars and firmness up to 5 d shelf life [66].

The use of chitosan as an inducer of resistance is definitely of great significance considering the systemic and resilient nature of defence proteins in plant tissues when elicited, and this is important towards delaying the resumption of an infection. Latent tissue that typically begins to activate when tissue resistance declines. In conclusion, chitosan has shown great potential as a natural substance, biodegradable, biocompatible without toxicity or side effects with antifungal activities with a direct effect on the growth and development of the fungus at the level of conidium and mycelium [64]. The information generated by transcriptome studies in chitosan-pathogen interaction systems indicates the effect of chitosan on metabolic pathways important for the defence of the fruit against the attack of the pathogen and in the fungus, resulting in morphological and biochemical alterations that inhibit the growth and germination of the pathogen.

Chitosan has an excellent fungicidal potential against the major fungi that cause damage to tropical fruits, such as C. gloeosporioides. Fungicidal ability of chitosan, proposes that the electrostatic interaction between the NH$_3^+$ groups of chitosan and the phosphoryl groups of the phospholipids present in the cell membrane of the fungi causes damage in this, resulting in an increase in permeability. In addition, it is possible that short chains of chitosan can pass through the wall and membrane and interact with the DNA and the RNA interfering with their function, losing functionality of the fungal structure. Its chelating effect could decrease the availability of some metals needed in enzymatic processes, inhibiting the process of the pathogenesis of fungi [37]. Alterations in the internal and external morphology of the conidia and mycelium caused by chitosan would provoke a biochemical-physiological stress.

**Aloe Vera Gel**

Aloe vera gel is one of the natural compounds that has gained great interest. It is mainly constituted of polysaccharides, and can easily make a uniform layer on the surface of the fruit when applied as an antimicrobial coating agent [67]. A bioactive coating was prepared using a pectin (1.1% w/v), candelilla (Euphorbia antisiphilityca Zucc.) wax (0.16%) and aloe vera mucilage (5% w/v) functionalised with Larrea tridentata purified polyphenols. The bioactive coating inhibited the growth of necrotic Botritis cinerea and Fusarium oxysporum was able to extend fruit shelf-life, keeping the organoleptic characteristics of avocado cv. Hass [55]. Candelilla wax is an FDA approved edible non-restrictive ingredient commonly used in the food and candy industries. Pectin is employed as biopolymer for coating because it is recognised as GRAS (CAS N° 9000-69-5), with the capability of stabilising other food ingredients, without limit restrictions other than current good manufacturing practices [55].

**Carboxyl Methylcellulose and Pectin**

Postharvest treatments containing carboxyl methylcellulose(CMC 1% w/v) blended with moringa leaf and seed extracts (2%) were used to treat ‘Hass’ or ‘Gem’ fruit. This CMC is a commercially known hydrophilic polysaccharide-based edible coating. Both moringa leaf and seed extracts exhibit significant antimicrobial effect on the growth of fungi. The image analysis revealed destruction of the hyphal structure for all pathogens, which was exposed to the extracts, while their respective control remained intact. The CMC containing moringa extract suppressed all postharvest diseases, thereby improving the shelf life of the avocado fruit during storage [67]. In a separate study, the effect of a pectin-based coating was evaluated on the kinetics of quality change in stored avocados at three
temperatures (5, 10 and 20 °C) [56]. The coating extended the shelf life to over a month at 10 °C by suppressing CO₂ evolution, weight loss and colour change [56].

Wax Coatings

The use of an edible film based on candelilla wax with a potent antioxidant ellagic acid on whole avocado was observed to control growth and spread of anthracnose, while preserving the appearance and overall quality during 6 weeks of storage [67]. Another important wax for potential use on avocados is carnauba wax, which stems from the leaves of the Brazilian palm *Copernicia cerifera*. Applications of Carnauba wax containing *Lippia scaberrima* EO improved the protection of cv. ‘Fuerte’ against anthracnose [59]. In both studies, the wax coatings could prevent fruit weight loss due to their barrier properties by reducing water vapour transfer between the fruit and the environment [42].

Biocontrol

Application of biological control agents offers another important viable disease control strategy in place of synthetic chemicals. This involves studying the naturally occurring epiphytes on the plant surfaces with the prime aim of manipulating them to attain disease control. Stem-end application of a cell suspension of *Aureobasidium pullulans* delayed disease incidence of unripe fruit by 2 days compared to controls, which had either stalk intact or not [67]. Increased chitinase and β-1,3-glucanase levels were retained at ripe stage of avocado fruit, pre-treated with the *A. pullulans* [68]. Elsewhere, bacterial extracts of *Serratia* sp. showed a high potential for the control of postharvest rot infections caused by *C. gloeosporioides* [69]. Yeast strains have also been shown to impart antagonistic benefits against avocado disease-causing pathogens. The yeast strains had been previously shown in vitro antagonistic activity against *C. gloeosporioides* [69]. Various *Bacillus* spp., which were originally isolated from leaf and fruit surfaces of avocados, were also observed to effectively control anthracnose of avocado [70,71].

Biocontrol agents are believed to compete for nutrients as their primary mode of action against fungal pathogens. Other mechanisms, involving the use of cell wall-degrading enzymes, production of antifungal diffusible and volatile metabolites, induction of host resistance and mycoparasitism have been cited in the bio-control activity of yeasts [72]. Although several antagonists of postharvest pathogens have been identified and tested in the laboratory, semi-commercial, and commercial settings, the full commercial potential has not been realised [73]. Very few bio-control antagonists have been commercialised due to difficulties in mass production constraints and inconsistent performance when used under commercial conditions [73].

2.5. Postharvest Phytosanitary Treatments

The postharvest insect infestation also significantly affects the economic losses. Avocado seed moth (*Stenoma catenifer* Walsingham), false coding moth (*Thaumatotibia leucotreta* Meyrick), large avocado seed weevil (*Heilipus Lauri* Boheman), red-banded thrips (*Selenothrips rubrocinctus* Giard) [74] and the oriental fruit fly (*Bactrocera dorsalis* Hendel, Diptera: Tephritidae) [75] are common pests affecting the avocado fruit quality. The impact of pest damage can be controlled by killing, sterilising, and or eliminating the pests [74]. Effective nonchemical pest control requires integration of biological, and physical, quarantine requirements [74]. ‘Cold disinfestation’ treatment was recommended for the South African avocado fruit industry. According to Ware and Du Toit [75], cold sterilisation at 2 °C for 29 days was effective for eradicating *Ceratitis capitata* (Wiedemann), *C. rosa* Karsch and *C. cosyra* Walker. Whereas 2 °C 18 d Cold sterilisation is required to eradicate *Bactrocera (invadens) dorsalis* (Hendel) [75]. However, cold sterilization at 1 °C revealed deleterious effect on fruit quality [76]. Although food irradiation technology was successfully implemented for different fruit commodities, and 150 Gy (minimum radiation dosage) has been approved in various commodities including avocados. However, irradiation technology it is not suitable for all avocado cultivars including the ‘Hass’ avocados due to induced skin injuries [75].
3. Future Perspectives

A lot of potential efforts still remain towards the postharvest preservation of avocados in a health-conscious manner. Electrolysed oxidizing water (EOW) is among the promising alternatives to synthetic chemicals for postharvest disease and pest management of avocados [77]. The EOW can be simply defined as ionised water solution or electro-activated water with a high oxidation-reduction potential essential for stripping away electrons from microbial cell membranes leading to cell wall collapse [77]. Overhead spray application of ‘Hass’ avocados with EOW (20% v/v) reduced the anthracnose incidence to 12%–35%, which was reasonably comparable to that of Graduate A+ fungicide control of 34% [77]. A reduction of 68%–85% vs. 90–100% in lesion size development of anthracnose was observed using EOW and Graduate A+, respectively [77]. Therefore, EOW shows potential to replace the currently used postharvest fungicide Prochloraz® application. Use of physical non-thermal fruit preservation can also be achieved by means of light-emitting diode (LED) or ultraviolet (UV) radiation [78]. ‘Hass’ fruit that had been UV-C irradiated for 15 and 20 min prior to storage at 10°C and 90% RH expressed a higher antioxidant activity, reduced weight loss and maintained soluble solids levels compared to the controls [79]. Furthermore, less energy is required when using UV compared to normal thermal treatments. More research should also be invested towards the formulation of more edible eco-friendly nano-coatings. Nanoemulsion coatings made from orange EO (70%) and xoconostle extract (10%) and food-grade mineral oil as the diluent were applied onto ‘Hass’ fruit prior to storage. The treatments retarded the PPO activity, reduced the weight loss and fruit browning, while maintaining the total phenol and flavonoid content up to 60 days storage period [80]. Fruit ripening of the avocados was also delayed compared to the control thereby increasing the postharvest life of the avocado fruit. Although the potency of fungicides remains superior to that of most biological compounds, when combined the biological compounds can be equally as effective. However currently there are no commercial biological agents available for the avocado industry and the application of biological agents are effective only during integrated treatments. Inducing defence mechanism via using elicitors can be beneficial to control postharvest decay and currently adopted as a novel approach to maintain fruit quality. On thus note use of light-emitting diode (LED) are becoming very poplar and by selecting the appropriate colour lights can be installed in cold storage facility to reduce the postharvest due to decay and disorders. Also, the shelf life of the fruit can help to extended on the shelf life to benefit marketers [81]. In addition, future research may also be focused on active slow release packaging films and coating emulsions that can consistently retain their secondary metabolites the phenolic compounds for prolonged periods. Avocado fruit losses stemming from uneven colouration can be alleviated using modified atmospheres enriched with 1-MCP or using preharvest treatments. On top of the targeted analysis of basic fruit quality attributes after different post-harvest treatments, the untargeted metabolomics approach can be conducted to provide in-depth knowledge of treatments effects of the overall fruit composition and quality.

4. Conclusions

To avoid health and environmental damage, maximum residue levels for fungicide and pesticide use have been set. Failure to comply with these specifications by producing countries will lead to product withdrawals by receiving markets. Investing in non-destructive technologies, such as NIR, to detect mechanical defects and decay and developing biodegradable packaging materials that can cushion avocado fruit from mechanical damage is important to prevent fruit loss during marketing. Based on this review it is evident that there is still a lot of room to develop postharvest management techniques using environmentally friendly and non-chemical approaches suitable for avocados in a safer and health conscious manner. Research into other avocado cultivars is also important to prevent the current overreliance on one cultivar alone by the global community.
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