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# Strawberry

Pre- and Post-Harvest Management Techniques  
for Higher Fruit Quality

*Edited by Toshiki Asao and Md Asaduzzaman*





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Strawberry - Pre- and Post-Harvest Management Techniques for Higher Fruit Quality

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Edited by Toshiki Asao and Md Asaduzzaman

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# Preface

Strawberry is the most popular fruit in the world and a good source of bioactive compounds, providing human health benefits. It also contains considerable amounts of vitamins, minerals, flavonoids, and phenolic acids. Strawberry is a luscious, delicate, and perishable fruit having a short shelf life. Thus, it needs careful management to maintain its quality. Preharvest production factors should also be considered for high-quality strawberry production. Fruit harvest practices and postharvest techniques of strawberry fruit greatly affect its quality.

This book mainly deals with pre- and postharvest management practices of the strawberry to ensure that high-quality fruits are delivered to the consumer. The influence of climatic variables, cultural practices, harvesting techniques, and use of chemicals and other natural compounds on fruit quality are discussed. Factors affecting fruit growth and development and processes regarding maturation and biochemical changes during fruit ripening are also presented in one of the chapters of this book. Some chapters provide information regarding harvesting, storing, packaging, transporting, and also selling that affect strawberry quality greatly. Enhancement of yield and antioxidant contents in the strawberry by various natural products, including chitosan and probiotic bacterial, are also included in this book. The final chapter states that antioxidants present in strawberry fruit play a dietary role in alleviating oxidative stress in experimental liver models.

Recent research work on nutritional quality, culture practices, and pre- and postharvest management techniques of the strawberry are brought together in this book to further the field of plant biological science. Publication of this book would have been impossible without the contribution of many researchers around the world. Our sincere acknowledgment goes to the authors who contributed their valuable research work to this book.

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# The Effect of Preharvest Factors on Fruit and Nutritional Quality in Strawberry

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## Abstract

Strawberries play an essential role in human nutrition and health, especially as a source of vitamins, minerals, and dietary fiber. They also have health-promoting compounds that lower the risk of cancer and cardiovascular disease. The nutritional value of strawberries varies greatly among cultivars. Traditional and molecular breeding techniques can be used to develop varieties with enhanced nutritional quality and improved flavor to meet consumer preferences. Climatic conditions, such as temperature and light intensity, and other preharvest factors, e.g., soil type, fertilization, irrigation, mulching, and other cultural practices, have a significant effect on strawberry fruit quality. Additionally, the extent of postharvest physical and physiological injury and potential fruit loss is affected by preharvest parameters. In this chapter, the effect of preharvest factors on fruit and nutritional quality of strawberry is discussed.

**Keywords:** strawberries, cultivar, genetics, environment, light, nutrition, temperature, soluble solids, acidity, firmness, fruit quality

## 1. Introduction

Several reports confirm significant (35–50%) loss in horticultural produce after harvest. These losses are mainly due to dehydration, decay, and physiological disorders during postharvest handling. Fresh products also undergo rapid changes in nutritional and sensory quality after harvest, some of which contribute to loss of market value [1]. Losses can be mitigated through better management of pre- and postharvest factors. Differences in postharvest loss of fresh produce between developed (5–35%) and developing countries (30–50%) as reported by Kader [2], Salami [3], Kitinoja [4], and Ray and Ravi [5] are due to the higher capacity and better infrastructure in developed economies for managing these factors [1].

In strawberry, preharvest management is a prerequisite for producing good quality fruit. Quality deterioration starts as soon as the produce is harvested, and postharvest factors can only maintain but not improve the quality. The growers' decisions and management directly affect the final market value of strawberry and its acceptance by the consumers [1]. Many preharvest factors affect fruit quality at harvest. In this review, we will discuss genetic variability, climatic conditions, and cultural practices that affect quality of strawberries.

Some review papers exist on the effect of preharvest factors on strawberry fruit quality [6–9], but the most recent one [9] addresses major temperate berry crops and does not focus solely on strawberry. Also, since then many new research papers have been published. Therefore, this chapter will review recent publications and elaborate on the effect of preharvest factors on strawberry fruit and nutritional quality.

## **2. Strawberry fruit quality**

Strawberry fruit quality is a composite of sensory characteristics (color, appearance, texture, mechanical properties, diseases, and defects) that give value and enjoyment to consumers [10]. Currently, strawberry producers and handlers are lengthening shelf life through early harvesting of firm fruit with less developed color. This process pays little attention to flavor, taste, and nutritional quality of harvested fruit and yet repeat purchases by consumers have been shown to depend on taste and eating quality of fruits. Therefore, the challenge is to encourage consumers to be willing to pay more for local fruit and varieties with higher quality (because they often require more careful handling and have lower yield), and producers and handlers to pay more attention to maturity, sugar content, taste and flavor of harvested strawberries [2].

Like other fresh produce, strawberry fruit quality is a product of the interaction between variety and preharvest factors. Preharvest factors such as climatic conditions and cultural practices determine the inherent quality of strawberries and the interaction of genetic characteristics, and preharvest factors determine the ultimate quality of the fruit [11]. Because strawberry fruit quality cannot be improved after harvest, the role of preharvest factors must be understood in order to improve consumer acceptance and shelf life [12]. This is because the ripening process in strawberries stops at harvest, and fruit should be picked when fully ripe to ensure good flavor and quality [13].

Maturity indices are important for deciding when to harvest strawberries. Color, soluble solids content (SSC), and titratable acidity (TA) are used as harvest indices in strawberries with >2/3 of fruit surface showing pink or red color, minimum SSC of 7% and maximum TA of 0.8% accepted as a minimum standard for timing of harvest [14]. Once strawberries ripen, they require quick marketing and careful handling to minimize injury and spoilage [13], and the indices provide some marketing flexibility to ensure that eating quality is attained by the time the fruit reaches the consumer while at the same time limiting postharvest losses. The necessity to ship strawberries to distant markets often results in harvesting at less than ideal maturity meaning less optimum quality for the consumer [13].

Berry fruits are extremely perishable and have a short market life. Several studies have shown that strawberry fruits harvested slightly under-ripe are firmer, have less decay and a longer shelf-life, and would ship better than fully ripe strawberries [15]. Because they are nonclimacteric fruit, strawberries produce very small amounts of ethylene and do not respond to ethylene treatment [13]. Therefore, ripened fruit should be kept at 0°C until time for display at retailers [14].

Sugars and organic acids have an important impact on the sensory quality of strawberry fruit. For example, a strawberry with very low sugar and acid content tastes flat [16]. Strawberry fruit contains reducing sugars such as fructose, glucose, and sucrose, comprising >65% of total fruit SSC [16]. Glucose and fructose are found in almost equal concentrations [17], while sucrose levels are generally much lower [18, 19]. The proportions of fructose, glucose, and sucrose are important in the perception of fruit quality since fructose is 1.8 times sweeter than sucrose, [20] and sucrose is about 1.7 times sweeter than glucose [16, 21].



Citric acid forms the major organic acid found in strawberry fruit [22], representing 88% of the total organic acids in ripe fruit [23]. Malic acid is the second most prominent organic acid in the fruit [23], and the organic acid level (malic + citric) was found to be positively correlated with TA [16].

The major phenolic compounds in strawberries include anthocyanins and proanthocyanidins, ellagitannins and ellagic acid conjugates, cinnamic acid conjugates and hydroxycinnamic acid derivatives, catechin, flavan-3-ols, flavonols, and flavons [24]. These compounds have numerous health benefits [25] such as antioxidation and anti-inflammatory activities [26, 27]. Also present in the strawberry fruit are polyphenols or tannins, which are responsible for astringency [19, 28].

Nutritive composition of sugar, organic acid, and phenolic compounds in strawberries is very diversely distributed. To obtain the best nutritive values, the interaction between genotype and environment not only needs to be optimized, ideal harvest and post-harvest storage conditions must also be maintained.

### **3. Genetic variation**

Results indicate that the effect of genotype on strawberry fruit and nutritional quality is stronger than that of growing conditions [29]. Strawberry cultivars vary greatly in their rate of softening and overall texture [30]. It has been found that genetic factors have a direct influence on strawberry texture with environmental factors acting only to modify the expression of textural traits [30].

Studies have shown that that genotype affects total organic acid content [31], while vitamin C content in strawberry varies among cultivars and between tissues. For example, Nelson et al. [32] found a range from 19.3 to 71.5 mg/100 g ascorbic acid in six strawberry cultivars from four locations [33], while Ezell et al. [34] found a higher rate of 38.9–88.9 mg/100 g in 28 named varieties and 16 numbered selections. Nelson et al. [32] and Ezell et al. [34] reported an average of 45 and 60 mg/100 g, respectively with Ezell et al. [34] concluding that the ascorbic acid average of 60 can be increased to 80 or more through breeding. However, malic acid concentration appears to be independent of genotype [31].

The main anthocyanin found in strawberries is pelargonidin 3-glucoside, with cyanidin 3-glucoside and pelargonidin 3-rutinoside present as minor components [35]. Differences have also been reported for other quality attributes. For instance, Anagnostou [36] reported that fruit from the cultivar “Fern” had better color and more anthocyanins than from “Selva.” When evaluated for firmness, “Carlsbad” was the firmest and “Rosalinda” the softest [37], confirming that firmness is mainly cultivar dependent [36]. In the same study, Anagnostou [36] also found that TA was not significantly affected by cultivar. Differences in the incidence of albino fruit production as reported by Sharma [38] can be attributed to genetic variability among cultivars.

It has also been shown that relative distribution of phenolic compounds varies with genotype. Up to a 4-fold difference in flavonol content was observed between cultivars but with only slight variations associated with growing environment [39].

These results suggest that different cultivars can be used for different purposes. Some such as “Toyonoka” are firmer and more suitable for distant markets but may have lower vitamin C, anthocyanins, phenolics and flavonols, others like “Oso Grande” with good nutritional values are suitable for fresh consumption, while some like “Mazi” with high anthocyanin and flavonol content but lower levels of vitamin C, citric acid, and total soluble solids (TSS) may be valued for their functional properties [35].

Nutritional quality can be considered an inheritable trait that can be improved through breeding [29], and breeding and biotechnology programs are working to produce new varieties with improved fruit and nutritional qualities combined with

high plant production efficiency [29]. Wild species like *F. virginiana* spp. *glauca* and *F. vesca* are good sources of bioactive compounds. *F. virginiana* spp. *glauca* is also an important genetic source of nutritional quality and other unique traits such as day neutrality, and plant and disease resistance [29]. Breeding for improved nutritional and fruit quality parameters offers the possibility of new commercial varieties that can yield high-quality fruit at reasonable cost [29].

#### 4. Climatic conditions

Environmental conditions seem to have a major influence on flavor compound formation in strawberry. Watson [19] reported that SSC in strawberry was more dependent on environmental conditions during production than on the genetic makeup of the plant. Furthermore, relating growth conditions to flavor data may allow modeling of plant and fruit responses to the environment and provide a powerful tool to growers and retailers to manipulate fruit quality [19].

Below are environmental factors that affect fruit quality in strawberry.

##### 4.1 Light intensity

Preharvest conditions such as light intensity can affect strawberry fruit quality and phytonutrient content. Light is required for proper leaf and fruit development and can improve the fruit quality, but light above photosynthetic saturation levels, especially intense exposure, can increase the fruit temperature and may result in fruit damage and a loss of quality [30, 40]. On the other hand, insufficient light typically results in smaller strawberry fruits [41]. In strawberry, low light decreases the surface glossiness of the fruit [41] and reduces color development [11, 38].

Ezell et al. [34] concluded that bright sunny weather favored high ascorbic acid content, while cool, wet weather resulted in low values. This confirms an observation by Wang [12] that strawberries grown at higher light intensity had increased levels of ascorbic acid. Even fruits shaded by foliage or ripened on cloudy days had 10% less ascorbic acid than berries exposed to sun [42], while berries shaded by leaves showed little change between cloudy and sunny days. Ezell et al. [34] also reported that everbearing varieties grown during the long, warm days, and intense light of early June averaged 34% more ascorbic acid than did the same varieties in late September. By shading either or both berries and plants, Hansen and Waldo [43] found that unshaded berries contained 13% more ascorbic acid than did the shaded ones and 68% more than when plants and berries were shaded. Unshaded plants produced higher dry matter in fruits at the expense of leaf growth but not fresh weight implying fruits with lower moisture content [44].

Light intensity, affected firmness, TSS, acidity, and anthocyanins [36]. The effect of shading was not significant for phenolics, but the opposite was observed for anthocyanins. Shading of strawberry plants has also been shown to cause significant reduction in the concentration of flavor compounds (hexenal, hexanal, ethyl methyl butyrate, and methyl butyrate) in fruit [19].

Light also influences anthocyanin synthesis and therefore color formation in fruit [45]. It appears that lower light intensity favors the development of albinism in strawberry with Sharma et al. [38] reporting that strawberry plants grown under low light intensities (shade) tend to produce a higher proportion of albino fruit.

Production methods will affect the amount of light to which a crop is exposed. Solar radiation experienced by crops grown in a polythene tunnel with new plastic may be 10% less than an outdoor crop, and a glasshouse could reduce light levels by 30% or more compared to that of an outdoor crop [46].

## 4.2 Light quality

The color of plastic mulches frequently used in raised-bed culture affects fruit quality. The most commonly used plastic mulch color is black [47]. Berries that ripened over red plastic mulch were about 20% larger, had higher sugar to organic acid ratios, and emitted higher concentrations of favorable aroma compounds. It has been said that the ratio of far red (FR) to red (R) light reflected from the red mulch modified gene expression through plant phytochrome and increased fruit size, phytonutrient concentrations, flavor, and aroma compounds [47].

Strawberry (cv. Toyonoka) fruit color was greatly affected by light quality under different colored filters (green, neutral, yellow, blue, to red light). Fruit color (chroma), ascorbic acid, yield per plant, and fruit size improved with increasing exposure to reddish orange color. All fruit quality parameters measured (e.g., color, sugar, acid) were negatively affected by green color [48].

In another study using five different mulch colors (red, blue, yellow, green, black, and silver), red mulch gave results similar or better than black mulch. Silver mulch reduced fresh fruit weight, fruit length and leaf area, while red mulch increased it. Silver mulch also reduced pH, ratio of TSS/TA and fruit dry weight, while black mulch increased the ratio. It is thought that increased light from the red and far-red spectrum reflected from red mulch is absorbed by phytochromes resulting in improved plant growth and fruit quality [49].

## 4.3 Temperature

Plant growth and development is largely affected by temperature. Temperature also affects cellular compounds and their structure, which ultimately affects firmness. Lower temperatures during the growing season increased fruit firmness [36], and growing strawberry under different temperatures (day/night) could also affect antioxidant activity and total flavonoid content. High temperature growing conditions (25/30°C) significantly enhanced antioxidant activity, as well as anthocyanin and total phenolic content [12]. Wang et al. [51] also reported that “Kent” strawberries exposed to warmer nights (18–22°C) and warmer days (25°C) had higher antioxidant activity than berries grown under cool day and night temperatures (18/12°C). In a separate study, Moretti et al. [50] found that high temperature conditions significantly increased flavonoid levels and consequently antioxidant capacity. Ascorbic acid content in strawberries is also highly affected by climate conditions and growing area [12]. Moretti et al. [50] showed that higher day and night temperatures have a direct influence on strawberry fruit color with berries ripened under these conditions being redder and darker.

However, Wang et al. [51] showed that in strawberry, fruit temperatures can exceed air temperatures by as much as 8°C on sunny days. High fruit temperatures could inhibit enzymes, such as sucrose synthetase, which acts on sucrose production. Increased fruit temperatures may also induce a higher transpirational flux within the fruit [51].

Freezing temperatures on the other hand can be detrimental to strawberries. A radiative freeze typically occurs under clear skies with calm or light wind, and a relatively high subfreezing temperature or dew point (similar to the conditions that often cause frost). Radiative freeze damage of strawberry often results in smaller fruit, and depending upon the developmental stage when damage occurs, misshapen fruit are produced [11].

## 4.4 Climate change and elevated levels of carbon dioxide and ozone

Although the climate change subject is controversial, its potential impact on agriculture continues to be discussed. However, few studies have considered the

potential impact of climate change on fruit and vegetable quality after harvest [52]. Temperature increase and the effects of greenhouse gases are among the most important issues associated with climate change. Beside rising temperatures, climate changes are also a consequence of changes in the composition of gaseous constituents in the atmosphere. Carbon dioxide accumulation in the atmosphere has direct effects on postharvest quality [50].

The highest temperature that strawberry fruit mature normally is 35°C. At high temperatures and elevated CO<sub>2</sub> levels, carbohydrates, such as starch and soluble sugars are degraded in the respiration process and the proteins and most minerals decrease. The nutritional quality also decreased due to more phenols and ascorbic acid. However, the effect of temperature is more pronounced than the elevated CO<sub>2</sub> levels [52].

Elevated CO<sub>2</sub> levels in storage slightly increased dehydroascorbic acid and firmness, prevented ascorbic acid reduction, and reduced anthocyanin, flavonoids, antioxidant activity and total phenolic compounds [53]. In contrast, increased CO<sub>2</sub> concentrations in the growing atmosphere (300 and 600 μmolmol<sup>-1</sup> above ambient) resulted in increased anthocyanin and phenolic and ascorbic acid content [54]. Siriphanich [55] also reported increased firmness in strawberry treated with CO<sub>2</sub>. Cell wall analysis showed lower water-soluble pectin and higher chelating soluble pectin in CO<sub>2</sub>-treated strawberries. The mechanism of firmness enhancement by CO<sub>2</sub> was possibly due to changes in intercellular pH and its solute composition.

The influence of ozone on strawberry depends significantly on cultivar and susceptibility to oxidative stress. The effect of ozone on vitamin C content is variable in the reviewed articles and mostly cultivar dependent. In “Korona” and “Elsanta” tested by Keutgen and Pawelzik [56], ozone caused a decrease in ascorbic acid content, and lowered fruit sweetness. The ozone stress did not influence yield, size, antioxidative capacity, anthocyanins, or phenolic compounds of fruit. In the more sensitive cv. “Elsanta,” ozone induced sepal injuries and fruit impairment, and a decrease in glutathione content. In contrast, fruit quality of the less sensitive cv. “Korona” remained almost constant [56].

In cv. “Camarosa,” ozone enriched storage (0.35 μL/L) for 3 days, increased vitamin C by three times, and reduced volatile esters 40% compared to control [57]. On the other hand, Moretti et al. [50] reported that strawberries stored in atmospheres with ozone ranging from 0.3 to 0.7 μL/L showed no effect on ascorbic acid levels after 7 days of storage under refrigerated conditions.

## **5. Cultural practices**

### **5.1 Soil and soil amendments**

Soil types, fertilization, composts, and mulching influence the water and nutrient supply to the plant and can affect the nutritional composition, ascorbic acid content, and antioxidant activity of harvested fruit [16]. Plants grown in low-organic-matter and low-cation-exchange-capacity sandy soil amended with Ca, magnesium (Mg), and N produced more ascorbic acid in fruit than plants without supplemental fertilizer [12]. Also, Wang and Lin [16] reported that strawberry organic acids, malic acid and citric acid, were increased by the addition of fertilizer [16].

Composts have been utilized in agriculture as a significant source of organic matter. As a soil supplement, compost significantly enhances plant growth, fruit quality, and ascorbic acid and flavonoid content in strawberries. Compost causes changes in soil chemical and physical characteristics that increase beneficial microorganisms, and nutrient availability and uptake thus favoring plant growth.

Strawberry plants grown with compost yielded fruits with high levels of phenolics, flavonol, and anthocyanin content. The free radical absorbance capacities for peroxy, superoxide, hydrogen peroxide, hydroxyl, and singlet oxygen in strawberries increased significantly with increasing compost use [12, 16]. Also, compost significantly increased levels of organic acids (malic and citric acid), sugars (fructose, glucose, and total sugars), soluble solids content, and TA content in “Allstar” and “Honeoye” strawberry cultivars [16].

Strawberries grown organically showed higher levels of total phenolics compared to those produced by conventional agricultural practices, mostly because they received all required nutrients from organic matter. Also, flavonol content was higher in organically grown than in conventionally grown strawberry fruits. These data provide evidence that an improvement in the antioxidant defense system of the plant occurred as a consequence of the organic cultivation practice [12]. It has been shown that N can become a growth-limiting nutrient in organic production [39].

Strawberries need nearly neutral (6–6.5) soil pH and variation from this range affect mineral uptake and plant growth, development, yield, and fruit quality [58]. Increasing soil pH significantly reduces uptake of minerals such as iron and manganese [58, 59]. Iron deficiency has been observed in many crops when grown in high pH calcareous soils. In these soils, iron deficiency is the most important abiotic stress limiting strawberry production [60]. In fact, iron deficiency results in extensive fruit abortion with a consequent reduction of yield and fruit weight [61].

However, although most studies show a positive correlation between organic production and crop performance in strawberry, Hargreaves [59] found that organic amendments did not increase fruit quality compared to inorganic amendments, and no differences in total phenolic content were observed between conventionally and organically grown strawberries.

## 5.2 Substrates

Strawberry is commonly produced in open fields, glasshouses or plastic tunnels. Pests and diseases gradually build up in soils and limit strawberry culture. Therefore, the use of alternative substrates (soilless culture) is a common cultural method in strawberries, especially in protected environments, and has been shown to improve fruit yield and quality [62].

Physical and chemical properties of substrates exhibit direct and indirect effects on plant yield and fruit quality. Different substrates such as peat moss, coconut coir, perlite, rockwool, and pine bark have been used. However, peat has been the best substrate for hydroponic culture [63].

Cheaper alternatives to peat are being explored. For example, Zeolites (alumina silicate crystals) that have a negative charge, high cation exchange capacity, and high water holding capacity have been tested. Djedidi et al. [64] reported improved yield and quality in tomato grown in a mixture of zeolite and perlite, while Fotouhi Ghazvini [65] reported improved yield but decreased fruit quality in strawberries in similar substrate.

## 5.3 Mineral nutrition and salinity

An important determinant of fruit quality is the availability of essential nutrients during growth and development. Among them, calcium (Ca) slows down the ripening and senescence processes in many fruits including strawberry [66]. Nestby et al. [67] and Prange and De Ell [9] report that research conducted over the past 25 years on the effect of Ca on postharvest quality of strawberry has provided contradictory conclusions. In some studies, foliar applications and soil amendments

of Ca did not affect fruit quality, regardless of cultivar, yet other studies have shown increased shelf life due to Ca application [66].

Furthermore, Singh et al. [66] reported that preharvest Ca sprays greatly influenced fruit color during storage. In storage, fruit harvested from control plants turned darker whereas from the Ca spray treatment were comparatively brighter. Similarly, higher values of hue (a) and chroma (b) in such fruit indicate that fruit receiving Ca were redder and more vivid. Strawberries soften considerably during storage as a result of degradation of middle lamella, cell wall, and cortical parenchyma cells. By extension, cell wall strength, cell to cell contact and cellular turgor, which are greatly influenced by Ca may affect fruit firmness. Thus, fruits which received Ca were firmer even after 5 days of storage [66].

Calcium deficiency represents one of the most common issues for strawberry growers. Calcium plays a role in cell division and the maintenance of cell permeability and cell integrity, all of which directly influence factors such as firmness and shelf life [66]. Strawberry fruits from Ca-deficient plants are small, hard textured, acidic, seedy, or with patches covered densely with achenes, with increased deformity [17]. Calcium sprays increase fruit firmness, vitamin C and shelf life during storage [68].

However, there are reports that increasing Ca application did not affect sensory quality and decreased TA and TSS of strawberries. Calcium increased firmness and storage life up to 900 ppm. Higher concentration seems to create toxicity and imbalances of other minerals in leaf tissue.

The form of Ca applied also changes the result. Application of gypsum at planting time did not influence fruit Ca content. Therefore, cultivar, form of Ca applied, and environmental factors must be considered during Ca fertilization of strawberries [68].

Nitrogen is another important nutrient for fruit quality with both positive and negative effects of N on yield and fruit quality reported. The effect of N on fruit chemical components is inconsistent and varies from year to year, depending on time and rate of application and other environmental factors [67]. Recommended optimum concentrations of leaf N for high-yielding strawberry fields in California, North Carolina, northeastern United States, and Ontario, Canada ranges from 2.0 to 4.0%, with samples collected mostly from the leaf blade [69–73]. Form of N also affects fruit quality. Ammonium up to a ratio of 1 to 6 nitrate increased fruit firmness, but reduced red color compared to nitrate alone. Fruit vitamin C, pH, and TSS decreased with addition of ammonium up to a ratio of 1:6 (ammonium: nitrate) and then returned to the original level at the ratio of 1.5:6. Ammonium reduced TA at all concentrations [74].

Tabatabaei [40] demonstrated that both high concentrations of  $\text{NO}_3$  (100%) and  $\text{NH}_4$  (75%) has an inhibitory effect on growth of strawberry, and it becomes more pronounced in the shaded conditions.

While low concentration of ammonium improved plant growth, higher concentrations, reduced growth due to the high demand for carbohydrates for ammonium detoxification and low nitrate concentrations.

Another possible explanation is that a high concentration of  $\text{NH}_4$  reduces the uptake of cations such as  $\text{Ca}^+$  and  $\text{K}^+$  and increases the concentration of  $\text{NH}_4^+$ , which is toxic for the plant [40].

Higher concentration of  $\text{NH}_4$  significantly reduced postharvest life probably due to Ca deficiency in fruit. Calcium deficiency associated with  $\text{NH}_4$  nutrition can induce loss of membrane integrity which in turn may lower the concentration of potassium (K) and Mg and influence the function of chloroplasts and mitochondria. In addition, higher rates of organic acid synthesis because of  $\text{NH}_4$  nutrition may immobilize Ca and Mg within the roots. Ammonium also reduces the uptake of Ca and K by the roots, and is used only sparingly or not at all during the early growth of crops under poor light conditions [40].

The general conclusion is that proper N fertilizer application could be effective in improving fruit quality. Plants with low or moderate vegetative growth tend to have firmer fruits. Higher N doses decreased fruit size and increased pest and disease occurrence and fruit rot and malformation. Petiole sap nitrate testing is a standard method that determines the N requirement at different developmental stages of growth and would help to reduce uncertainty of time and amount of N application [67].

Imbalances in certain macronutrients can also have a pronounced effect on shape, and size [11]. Magnesium and phosphorous deficiency tend to decrease flower and fruit size and increase the incidence of albinism. Potassium-deficient plants may produce shriveled fruits with brown calyxes, fruits fail to develop full color, and have pulpy texture, and insipid taste [67].

Among microelements, boron (B) has a direct effect on fruit quality. For example, B deficiency causes distorted flowers and fruits, reduces fruit size and number, and increases malformation [11, 67]. The influence of B on phenol metabolism has also been well studied [39]. Fewer malformed fruit in plants receiving B can be correlated with a higher concentration of B both in leaves and fruit [66], which may be due to the significant role of B on pollen germination and pollen tube growth.

Studies conducted by Sing [66] indicate that B application does not influence fruit quality in strawberry; however, B deficiency usually results in poor accumulation of TSS, and ascorbic acid. Other microelements also have roles in plant growth and development and are needed at lower concentrations compared to macronutrients. Iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and molybdenum (Mo) are needed at 40, 25, 20, 3, 0.4 ppm per dry matter of leaves, respectively [67]. No major effects of Fe, Cu, and Mn have been reported on fruit quality [67]. In general, balanced fertilization has an important effect on both fruit yield and quality.

Fruit quality has been observed to increase with saline solute concentration. A solution strength of  $1.3 \text{ mS cm}^{-1}$  EC is preferred during the spring season whereas  $2.2 \text{ mS cm}^{-1}$  EC proved to be best in the winter in terms of fruit quality. Fruit ripening was accelerated in salt-stressed plants [75], and salinity had no effect on fruit number, but decreased fresh and dry weight [44]. In three different studies on the effect of salinity on two strawberry cultivars differing in their sensitivity to NaCl stress: cv. Elsanta (sensitive) and cv. Korona (less sensitive) [76–78], salinity decreased total soluble solids and sugar (especially sucrose) content. Fruit quality, characterized as taste, aroma, and texture by a panel decreased significantly in “Elsanta,” but not in “Korona” [76, 78]. The more tolerant cv. Korona was characterized by an increase in reduced glutathione and better fruit taste [78].

Salt stress increased the antioxidant capacity, antioxidants pools (ascorbic acid, anthocyanins, superoxide dismutase) and selected minerals such as  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{K}^+$ , N, P, and  $\text{Zn}^{2+}$ , as well as lipid peroxidation in both cultivars [78], and also free and essential amino acid content, especially in cv. Elsanta. The ability of “Korona” to retain  $\text{Cl}^-$  in the root system more effectively than “Elsanta” resulted in 41% less leaf  $\text{Cl}^-$  at the highest salinity level and better growth under NaCl stress leading to relatively higher fruit yield and quality [76].

#### **5.4 Irrigation**

Strawberry plants need frequent irrigation due to a shallow root system, large leaf area, and fruits with high water content [79]. In a field study, it was found that irrigating at 200 hPa resulted in higher yield and better fruit quality when compared with irrigating at 300 hPa. Decreased fruit firmness and increased fruit size was also observed in irrigated plots [80, 81]. Particularly in light soils, irrigation significantly increases fruit yield and quality, and creates an opportunity for intensive

strawberry production. Both sprinkler and drip irrigation systems produced the same result, but water use was considerably lower with drip irrigation [82].

When strawberries are exposed to long-term water stress, leaf, crown, and runner growth are decreased. Fruit number and weight (yield) also decreases even as fruit maturity is accelerated [83]. Therefore, strawberries are generally considered unfit for deficit irrigation. However, mild deficit irrigation has been tested as a method to limit water use, and depending on the cultivar, to increase the concentration of some taste- and health-related compounds. For example, [84] report that deficit irrigation reduced berry size but increased dry matter content, sugars and acids in cvs. Elsanta, Sonata, and Symphony but not in cvs. Florence and Christine.

## **5.5 Cultural systems**

Strawberries are commonly grown on raised-beds using different polyethylene mulches. This system keeps the fruits clean, eases harvesting, saves water, and lowers herbicide use. Moreover, in Europe, strawberries grown on plastic mulches ripen a week earlier than those without mulch because of higher soil and canopy temperature, and reflection caused by the mulch may improve light conditions [39].

Significantly higher phenolic content has been reported in fruit from strawberry plants grown on plasticulture than in those grown in matted row culture. Additionally, higher anthocyanin content has been observed in strawberry fruits grown on plastic mulch compared to those grown on straw mulch. The elevated temperature inside the plastic may explain the higher phenolic content. White mulch increased the contents of the total phenolics and ellagic acid and the antioxidant activity in strawberry fruits more than brown mulch, whereas the total anthocyanin content was highest in fruits grown on brown mulch. Strawberries grown on red plastic mulch have been found to contain significantly higher amounts of aroma compounds than those grown on black mulch, which may be due to different light conditions caused by the mulch color [39].

Cultural systems affect plant metabolites such as total phenolic compounds, anthocyanin, and antioxidants. Hill plasticulture systems significantly increase total phenolic compounds, anthocyanin, antioxidants, and flavonoids compared to matted row systems. Cultural systems affect the quantity and quality of light reaching the plant and therefore, plant and soil temperatures, and soil moisture content. These conditions will affect plant growth and development and subsequently plant metabolites such as those mentioned previously [12, 51].

## **5.6 Planting date**

Planting date in glasshouse production affected phenolic content, and a statistically significant interaction was found between planting date and fruit order [39]. The crop from the latest planting date seems to have the highest total phenolic content and antioxidant activity, whereas anthocyanin content was lowest. In a related study, it was found that harvest date affected the ellagic acid content, with prevailing temperature and rainfall during harvest assumed to cause the differences [39].

Fruit quality did not change with the cultural cycle (summer-spring versus fall-spring), but the berries harvested in the spring had higher vitamin C and sucrose content and lower nitrate content compared with berries harvested in the winter [79]. Several works have demonstrated flavor differences between crops grown during different seasons [19]. Planting date affects plant mineral content due to changes in nutrient solution strength and light intensity. At lower light intensity during winter, growers increase nutrient solution strength, which can lead to higher fruit nitrate content compared to the spring. This can be considered undesirable, since nitrates may pose a potential threat to human health [19].



## 5.7 Maturity

Chemical composition in strawberry fruit varies by genotype and maturity. Following the pink stage, many phytonutrients are synthesized in parallel with the overall development and maturation of the fruit.

Harvesting at optimal maturity is essential for ensuring good fruit quality since important changes in individual sugar and acid content occurs in the final stages of ripening.

Antioxidant capacity also varies considerably with maturity. Many phytonutrients are synthesized in parallel with the overall development and maturation and strawberry fruit harvested at ripe stage consistently yielded higher antioxidant values than those harvested at the pink stage [12, 16]. Strawberries have the highest oxygen radical absorbance capacity (ORAC) values during the green stages [15, 16], while fruit harvested at pink stage (50% maturity) had the lowest ORAC and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity.

The concentration of total phenolic compounds is highest at the green stage and reduces during prolonged storage and processing. Ellagic acid concentration is highest in the early stages of fruit maturation (green strawberries) and gradually declines with ripening [85].

Ascorbic acid content increased from green to the pink stage by about 95% and then did not change until berries were overripe, when it then decreased. Ascorbic acid is lower in the inner than in the outer part of the fruit.

Overall quality and firmness of fruit harvested at the red ripe stage declined more rapidly than the white tip stage. Also, fruits were lighter and less red, reflecting less anthocyanin accumulation. Therefore, fruits harvested at three quarters red ripening stage can be stored for longer periods with better color and firmness than the fruit harvested when fully red. Strawberries harvested at early stages of color development (white stage) can become red during storage similar to commercially ripe fruit [15, 86]. This indicates that strawberries harvested at certain stages of maturity can synthesize pigment during storage under favorable conditions that are temperature dependent [45, 86] and can occur in darkness, but light can slightly increase the rate of pigment formation [45].

## 5.8 Harvest time and method

There are large variations in flavor quality between fruits per harvest and also from harvest to harvest. In strawberry, primary fruits are larger and ripen first and have less competition for resources. Therefore, there is a variation between primary, secondary, tertiary, and quaternary fruit in flavor and nutrient content. At each harvest, fruits from different stage of maturity and ripeness will increase the variability.

Different harvests during the season may even have more variability than fruit ripening stage, because, environmental conditions such as light may alter flavor compounds as well. Previous data from tomatoes Anagnostou [36] suggested a strong correlation between juice sugar and solar radiation across one season. The results show the difficulty in interpreting data where there is a variable light integral as well as differences [36].

Soluble solids and sugars decreased as the harvest season progressed, while TA, ascorbic acid content and anthocyanins increased [36]. A steady decrease in SSC and sugars as the harvest season progressed was noticed, most probably because plants were exhausted. Low levels coincided with peak production, while the highest values were noticed during low and early production (January) periods characterized by very low light intensity and short days. Titratable acidity increased from January to June, in parallel with increases in day length and light intensity [36].

Seasonal factor and environmental conditions affect fruit content and quality very strongly.

Organic acids that affect flavor (citrate and malate) and ascorbic acid were significantly higher in the spring than winter. This could be due to the higher carbohydrates as the precursors for ascorbic synthesis. Carotenoids are also available in strawberries, although less abundant than ascorbic acid. Unlike ascorbic acid, the amount of carotenoids is higher during winter than spring. This suggests that carotenoid synthesis is dependent on the interaction between temperature and light intensity [79].

The method of harvest and handling operations can determine the extent of physical injury and maturity variation, which both affect the nutritional quality of strawberries. Physical injury during harvest such as abrasions, cuts, and bruising can reduce the amount of ascorbic acid and increase fruit decay. Best harvest and handling practices should be implemented to reduce physical injury and maintain fruit quality [33].

### 5.9 Pollination, fruit order, and crop load

Inadequate pollination creates distorted strawberry fruit at maturity due to inadequate fertilized ovules, which secrete hormones necessary for fruit cell division and enlargement [11]. Insect pollination results in uniform strawberry fruit and bee (*Apis mellifera*) colonies are introduced into protected cultivations to pollinate flowers. Honey bees are not efficient in colder temperatures and other insects such as bumble bees are commonly used [87]. In Utah, field-grown strawberries pollinated by caged honey bees produced fewer malformed fruits than controls. Honey bees and large Diptera (*Eristalis* spp.) were the most common visitors to the strawberry flowers while sweet bees (*Halictus ligatus*) were the most efficient pollinators [88]. In Brazil, stingless bees (*Scaptotrigona* aff. *depilis* and *Nannotrigona testaceicornis*) work efficiently as strawberry pollinators in greenhouses [89].

Temperatures above 25°C can significantly affect pollen viability and germination and consequently yield and quality. However, certain strawberry cultivars produce heat-tolerant pollen, which in turn could result in higher fruit set [90, 91].

Fruit order significantly affected the phenolic content in fruits. In two separate experiments, the levels of the total phenolics, ellagic acid, and antioxidant activity were found to increase from primary to tertiary fruits. Fruit order caused at highest a 1.5–2.0-fold difference in phenolic acid content [39]. However, the highest anthocyanin content was found in secondary fruits.

Primary fruits are the largest and have more resources available for growth and protein synthesis and less available for the phenylalanine ammonium lyase enzyme to convert it to phenolic compounds. Another explanation is the dilution effect of larger cells with higher biomass in primary fruits [39].

In strawberry, the effect of crop load on fruit quality depends on the genotype. Low crop load is associated with higher total soluble solids in “Ventana” and “Candongia,” but not in “Camarosa” [92]. Crop load also increases over time meaning that even at similar light levels, the amount of assimilates available to individual fruits will change throughout the harvest period. The greatest demand for assimilates will occur during the development of secondary and tertiary fruits [19]. Crop load also affects firmness, TSS, acidity, and anthocyanin [36].

### 5.10 Chemical sprays and residues

Evaporation of surface water from overhead irrigation or chemical, fertilizer, and pesticide sprays create an unpleasant strawberry fruit appearance [11].

However, strawberries cannot be exposed to free water after harvest meaning residual chemicals on fruit surfaces cannot be washed away. Furthermore, pest control products may indirectly change fruit flavor and composition. For example, mite control improved sweetness and flavor intensity of “Sweet Charlie” strawberries but had no effect on fruit color or firmness [93]. There are many commercial products that affect plant growth and development, which may or may not be plant growth regulators. These products seem to enhance fruit set and growth, resulting in bigger fruit size and higher yields, or protect the plants and fruits from biotic and abiotic stresses [94].

Plant growth stimulators (seaweed extract, mixture of an auxin plus gibberellic acid, and nitrophenolates) increased fruit yield, size, and total anthocyanin concentration of strawberry cv. Camarosa, but did not change other characteristics (i.e., fruit juice pH, TA and TSS concentration, organic acid and carbohydrate concentration and fruit color). In a taste panel, the mixture of auxin and gibberellic acid received the best score [94].

In another experiment, strawberry plants were sprayed during fruit ripening and after 10 days with 2, 4 and 6 g l<sup>-1</sup> chitosan with no phytotoxic effect [95]. Chitosan increased firmness and reduced fruit decay, ripening rate, TA, and anthocyanin content during 4 weeks of storage. Formation of a chitosan film on fruit acts as a barrier for oxygen uptake thereby slowing the ripening process and modifying the atmosphere and ethylene levels without causing anaerobic respiration. In addition, chitosan coating can reduce desiccation by providing a moisture barrier as was reported earlier [95]. The reduced decay by chitosan is mostly related to delayed fruit senescence.

Plants receiving salicylic acid (SA) during vegetative stage through their nutrient solution produced firmer fruits with less weight loss, decay, and better quality [96]. Weight loss is due to metabolic activity, respiration and transpiration, and it is possible that SA decreased respiration rate and fruit weight loss by closing stomata. Salicylic acid as an electron donor produces free radicals, which prevents normal respiration. Pre- and postharvest application of salicylic acid significantly increased strawberry ascorbic acid content, TSS, TA and total antioxidant potential, and prevented fungal growth [97]. Increased ascorbic acid is mostly due to the increased activity of ascorbate peroxidase, [12]. Also, increased antioxidant ability and antistress power of plants and fruits induced by SA prevents vitamin C destruction. Salicylic acid decreased fungal development, although not fungicidal. However, activity of defensive enzymes (peroxidase, chitinase, and phenylalanine ammonia-lyase) increased in pear after SA spray. Conversely, rapid decrease in endogenous SA of fruits during ripening is simultaneous with rapid softening of fruits. It has been shown that SA affects cell swelling, which leads to higher firmness of fruits and prevents fruit softening (Wang [12]).

### **5.11 Silicon, and other natural compounds**

Recent studies show that silicon (Si) is a beneficial element for plant growth that plays an important role in plant resistance to biotic and abiotic stresses. Silicon improved strawberry plant fresh and dry weight, leaf area and relative water content, and yield under salinity stress. Both Si and salinity treatments increased acidity of fruit, but did not affect other fruit quality characteristics [98]. Silicon fertilization also reduced powdery mildew severity under high tunnel on all strawberry cultivars tested, and significantly increased yield of marketable fruits reaching as much as 300% with cv. Monterey [99]. A silicon-based wetter (Omex SW7) significantly increased the number and length of leaf hairs on both the upper

and lower surfaces of strawberry leaves and reduced the number of germinating powdery mildew (*Podosphaera aphanis*) ascospores and colonies. Moreover, potassium carbonate alone or mixed with Omex SW7 significantly reduced the number of germinating ascospores and colonies [100].

## 6. Conclusion

Several reports confirm significant (35–50%) loss of horticultural produce after harvest. In strawberry, preharvest management is a prerequisite for producing good quality fruit. Fruit quality and nutrient content of strawberries varies widely depending on the variety and environmental factors. Berry fruits are extremely perishable and have a short market life. Because strawberry quality cannot be improved after harvest, the role of preharvest factors must be understood in order to improve the shelf life.

Results indicate that the effect of genotype on strawberry fruit and nutritional quality is stronger than that of growing conditions and cultivars could be selected for maximum adaptability to the environmental conditions and market demand.

Environmental conditions seem to have a major influence on nutritional and flavor compounds in strawberries. Preharvest conditions such as light intensity can affect strawberry fruit quality and phytonutrient content. The color of plastic mulches frequently used on raised beds also affects fruit quality. Plant growth and development is largely affected by temperature, which affects cellular compounds and their structure and fruit firmness. Changes in the composition of atmospheric CO<sub>2</sub> and ozone constituents due to climate change may also affect fruit quality. Soil types, fertilization, composts, and mulching influence the water and nutrient supply to the plant and can affect the nutritional composition, ascorbic acid content, and antioxidant activity of harvested fruit. The use of alternative substrates (soilless culture) is a common cultural method in strawberries, especially in protected environments that has been shown to improve fruit yield and quality. Nitrogen and Ca are among the most critical elements for improving strawberry yield and quality. Strawberry plants need frequent irrigation due to a shallow root system, large leaf area, and fruits with high water content.

Cultural practices affect strawberry metabolites significantly. Higher phenolic content has been reported in fruit from strawberry plants grown on plasticulture than in those grown in matted row culture. Also, there is a large variation in flavor quality between fruits per harvest and from harvest to harvest.

Some of these environmental factors can be optimized in open fields according to the light and temperature conditions, such as planting date, mulch color and fertilization, although, their interactions may be hard to control. Other environmental factors can only be manipulated under protected cultivation. In general, to obtain reliable data on fruit and nutritional quality, when there is such a larger variation in growing conditions, other factors should be closely monitored.

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# Fruit Physiology and Postharvest Management of Strawberry

*Venkata Satish Kuchi and Ch. Sai Ratna Sharavani*

## Abstract

Strawberry is famous for its unique flavor and delicacy among the consumers all around the world. Nowadays, the concept of postharvest management is not only confined to preserving the nutritional attributes but also extended up to flavor that includes aroma. Strawberry is a nonclimacteric fruit and its short storage life and strategic sales in the market after harvest had compelled researchers to utilize technologies like cool store, modified atmospheric packaging, controlled atmospheric storage, different packaging systems, fumigation with nitric oxide, and diversified chemical treatments to preserve fruits for longer time. To apply or innovate new technology to extend life of strawberry fruits in the postharvest area, it is necessary to understand the physiology and biochemistry of fruits. This chapter reviews fruit physiology, recent trends, and future prospects in the postharvest management of strawberry.

**Keywords:** strawberry, postharvest, flavor, quality, packaging, storage, chemical treatment

## 1. Introduction

Strawberry (*Fragaria × ananassa*) belongs to family Rosaceae. It is cultivated throughout the world. Fruits have high vitamin C content which are consumed fresh. They are also processed into pastry or pie filling mostly. They provide great health benefits. Regular consumption of anthocyanins (found in berries) reduces the risk of heart attack. The antioxidants (like kaempferol, quercetin and anthocyanins) which are present in fruits reduce the formation of harmful blood clots which are associated with strokes. The antioxidants neutralize free radicals present in human body, inhibit tumor growth, and decrease inflammation in the body. Strawberry consumption decreases the risk of heart stroke due to high potassium content. For the above reason, strawberries were suggested to the people with blood pressure. It also counteracts the effects of sodium in the human body. According to the National Health and Nutrition Examination Survey, less than two of American adults meet the daily 4700 mg recommendation for potassium ( $K^+$ ). Strawberries are a smart fruit choice for diabetics as they have a lower glycemic index (40) than many other fruits.

Strawberries are low-growing herbaceous plants. Roots are fibrous in nature; basal leaves arise from crown, which are compound. Flowers are generally white, rarely reddish, are borne in small clusters on slender stalks arising from the axils of the leaves which look like the surface-creeping stems. As a plant ages, the root system becomes woody, vegetative propagation occurs from the “mother” crown which sends out runners (e.g., stolons) that touch ground and root. Botanically, the

fruit of strawberry is “accessory fruit” and is not a true berry. The flesh consists of the greatly enlarged flower receptacle in which many true fruits, or achenes, are embedded, which are popularly called seeds.

Strawberries are commercially cultivated both for immediate consumption and for processing as frozen, canned, or preserved berries or as juice. Due to the perishable nature of the berries and the improbability of mechanical picking, the fruit is generally grown near centers of consumption or processing and where sufficient labor is available. The berries are handpicked directly into small baskets and crated for marketing or put into trays for processing. Early crops can be produced under controlled conditions (glass or plastic covering). Strawberries are very perishable and require cool dry storage.

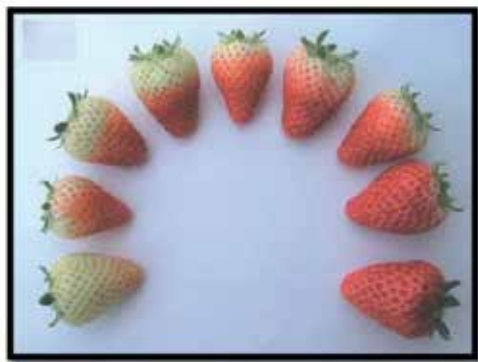
To innovate a new technology for extending storage life of strawberry fruits in the postharvest area, it is necessary to understand the physiology and biochemistry of fruits. This chapter reviews fruit physiology, recent trends and future prospects in postharvest management of strawberry.

## 2. Physiology and biochemistry of strawberry during ripening

Fruit ripening involves dramatic changes in the color, texture, flavor, and aroma of fleshy fruits. Both the palatability and nutritional quality of fruit are highly dependent on its consumption at an optimum stage of ripeness. However, ripe fleshy fruits are also perishable commodities, and this presents problems for fruit production, harvesting, storage, and marketing.

The general ripening programmes (**Figure 1**) displayed by strawberry typically include: (i) modification of color through the alteration of chlorophyll, carotenoid, and/or anthocyanin accumulation; (ii) modification of texture via alteration of cell turgor and cell wall structure; (iii) accumulation and modification of acids, sugars and volatiles that affect nutritional quality, aroma and flavor; and (iv) increased susceptibility to pathogens and herbivores [2]. These changes in flavor, color, aroma and texture make fruit ripening a complex process, which must be very tightly regulated. Fruit species are categorized as either climacteric or nonclimacteric, based on physiological differences in their ripening patterns [3].

Strawberry is a nonclimacteric fruit and fast growing, with a short postharvest life. During development, receptacle growth was due to a combination of cell division and cell expansion until seventh day after petal fall, and thereafter, only



**Figure 1.** Stages of fruit development [1], from left to right color development from tip toward receptacle (anthocyanin development).

cell enlargement occurs [4]. Accumulation of sugars, water and synthesis of cell walls were observed until 21–28 days after petal fall [5]. In strawberry and other fruits like grapes, growth may continue after initiation of ripening process [6]. Fruit enlargement continues until it reaches 25 per cent red or more, when chlorophylls have been completely degraded and anthocyanins begin to build up.

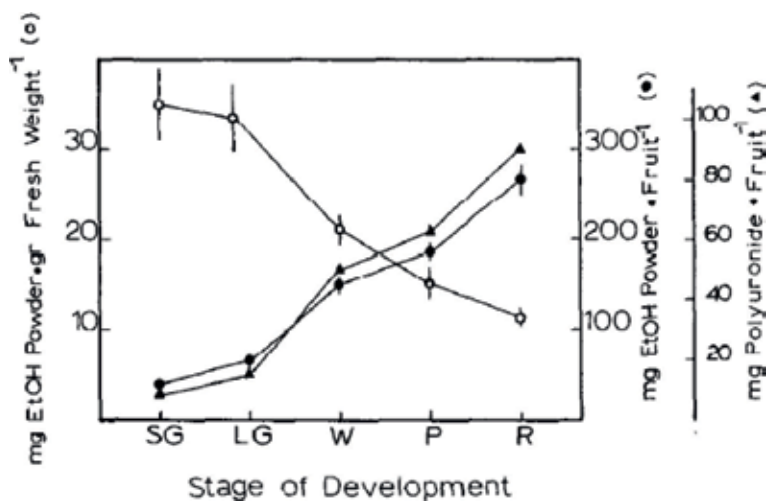
## 2.1 Role of hormones in fruit ripening

Auxin, the first plant hormone identified, may act as an inhibitor of ripening in some nonclimacteric fruits [7, 8]. In strawberry, it appears that auxin from the externally located achenes (seeds) inhibits the ripening of the fleshy receptacle [9]. Fruit development continues till the auxin level falls below the critical level in the receptacle and achenes, thus permitting ripening [10]. Therefore, removing the achenes promotes ripening, while treating strawberries with synthetic auxins delays ripening [11, 12].

## 2.2 Softening of fruit during ripening

During ripening, the primary cell wall of fleshy fruits shows structural and compositional change [11], which leads to loss of firmness and facilitates the attack of pathogens, enhancing postharvest decay and reducing the quality of fresh fruit. During ripening, water soluble polyuronides increases (Figure 2), whereas, there will be decrease in insoluble, covalently bound pectins. Concurrently, depolymerization of pectins occurs, which has been linked with the action of a number of hydrolases, mostly polygalacturonases (PG) [14]. Xyloglucan, the main component of hemicelluloses in dicotyledons, is also considered to play a vital role in cell wall structure, since it forms cross-linkages among the cellulose polymers. Xyloglucan endotransglycosidases, endoglucanases, and expansins contribute to the depolymerization of xyloglucans at the time of ripening [14].

Three major components of fruit organoleptic quality are flavor, sweetness, and acidity. Fruit with intense flavor also have high titratable acidity and high soluble solids [15]. Fruit soluble solids, sugars, titratable acidity, and organic acids at



**Figure 2.** Changes in polyuronides during fruit ripening [13], changes in ethanol insoluble powder, and total polyuronides during strawberry development (o) mg ethanol insoluble powder per gram fruit fresh weight (●) mg powder per individual fruit (▲) mg total polyuronide per individual fruit.

maturity are quantitatively inherited [16, 17]. Numerous biochemical changes are observed during strawberry development and especially during fruit ripening [18]. The major soluble constituents of maturing and ripe strawberries are soluble sugars and organic acids [6, 19].

### 2.3 Sugars

The major soluble sugars in strawberries are glucose [1.4–3.1% fresh weight (FW)], fructose (1.7–3.5% FW), and sucrose (0.2–2.5% FW) [6]. Glucose and fructose concentrations increase continuously during fruit development, while sucrose accumulates mostly during maturation [19].

### 2.4 Organic acids

The major organic acid is citrate, and its concentration ranges from 4 to 12 mg·g<sup>-1</sup> FW. This acid contributes greatly to fruit titratable acidity, which declines gradually during fruit development. The sugar/organic acid ratio is a major parameter of strawberry taste [6].

### 2.5 Amino acids

Of the other soluble constituents of strawberries, amino acids may also directly affect fruit taste, as was shown by the sensory evaluation of another fleshy fruit, peach [*Prunus persica* (L.) Batsch] [20]. Moreover, some amino acids are flavor precursors [20]. The major amino acids in strawberries are asparagine, glutamine, and alanine [21]. Anthocyanins (0.5–1.5 mg/g fruit weight) are a major component of the fruit, while ascorbic acid (0.3–1.2 mg/g fruit weight) makes an important contribution to the fruit nutritional value. Among the insoluble constituents, starch is present in young fruit and disappears before ripening [5].

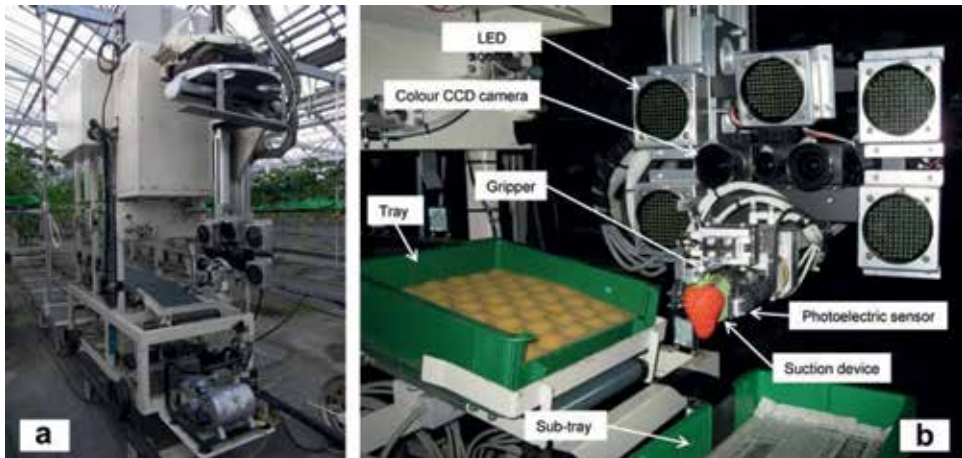
## 3. Harvesting

Harvesting is generally practiced after 3–4 months from planting. Strawberries are the sweetest when they are fully ripened on the plant. It is better to leave them on the plant for a day or two till they turn red. To ensure ripeness, taste test can be made. During harvesting berries, care should be taken as ripe ones bruise very easily. For harvesting, snap the stem just above the berry to remove them from the plant. Store harvested berries out of direct sunlight in some cool place, such as a refrigerator immediately after picking to increase the storage time. Strawberries can be consumed fresh or preserved by freezing or dehydrating and canning.

### 3.1 Robotic harvesting

The pericarp of a strawberry is so soft that workers must harvest the fruits carefully to avoid damage. The fruits are harvested early in the morning, before the temperature of the fruits rises and they become soft; workers need to select mature red fruits from among the many fruits that have set. These factors result in long working hours during the harvest period. Mechanical harvesting trials have been conducted on the assumption of once-over picking, but utilization of this strategy is not yet widespread [13]. The commonly used selective harvesting method requires high-tech and sophisticated robot technology. In short, it is essential to design an





**Figure 3.** Robotic harvesting in strawberry [1]: (a) high tech sophisticated harvester from a distance and (b) enlarged view with different parts of robotic harvester.

smart robot with human-like perceptive capabilities; for example, the machine would need to analyze fruit position, assess maturity level and pick the fruit without injuring the pericarp. Basic studies on robotic harvesting were initiated with orchard fruits [22]; since then, such studies have been ongoing in a number of countries [23]. This skill has then been used for vegetable fruits. Tillett [24] reviewed various robot prototypes and clarified the importance of the manipulator design and its application to practical use. Several studies have applied robotic technology to fields in greenhouses for instance, cucumber harvesting [25], strawberry harvesting (**Figure 3**) [1] tomato harvesting [26], aubergine harvesting [27] and de-leafing [28]. However, the performance and cost have not met commercial requirements.

#### 4. Precooling

Rapid removal of field heat from freshly harvested commodities is called precooling. It slows down ripening, respiration, senescence, decay, and water loss, thus helping for quality maintenance and prolonging shelf life [29]. Rapid precooling is most essential for produce such as strawberry which has a high rate of metabolism. The process of removal of field heat can be achieved by different methods that includes room cooling (RC), forced-air cooling (FA), hydrocooling (HC) contact icing, and vacuum cooling, each differing in efficiency of heat removal. Strawberries are typically cooled used forced air cooling. Delay in cooling of harvested strawberries results in reduction of number of marketable berries due to increased water loss, softening, and losses of sugars and vitamin C [30]. Thus it is usually recommended that strawberries should be cooled to temperatures near 0°C as soon as possible (within 1 h) after harvest to limit deterioration and decay [31, 32]. However, for commercial strawberry operations, this idea is rarely achieved due to factors such as the volume of strawberries handled, cooling and handling equipment availability, and capability, economics, energy, and market conditions. Hydro-cooling is a more rapid precooling method, but strawberries are not hydro-cooled commercially, due to decay problems by the water left on the berries after Hydro-cooling [29, 32]. Park et al. [33] proved that effectiveness for keeping the freshness of strawberries was best achieved by precooling at 4°C and storage at 4°C, respectively.

## 5. Postharvest treatments

### 5.1 Physical treatments

Physical methods include high or low temperature treatments, irradiation and use of modified or controlled atmospheres.

#### 5.1.1 High-temperature treatments

High temperature treatments can control insect pests, prevent pathogen infection, induce resistance to chilling injury, slow fruit ripening, and extend postharvest shelf life [34, 35]. Application of thermal treatments reduced the fungal development, ripening rate and extended the shelf life in strawberry [36]. Strawberry shelf life may be improved by an appropriate thermal treatment that could be used instead of fumigation to allow a more advantageous usage of this fruit in the commercial chain.

#### 5.1.2 Low temperature

Freezing of fruits and vegetable is one of the most common ways for maintaining the quality of these products. Frozen storage of strawberry, at 18°C after 7 months, had a specific effect on color but no significant different in total anthocyanin was observed [37]. Decrease of anthocyanin content in frozen storage strawberry, at 20°C after 6 months, depending on variety was 11–27.5% [38]. The storage temperatures of 18 and 24°C were best for preserving the qualitative characteristics (color, texture, flavor and wholeness) of the strawberries [39].

#### 5.1.3 Irradiation

Alternative control methods that do not leave residues, such as postharvest UV-C radiation, have been shown to prevent decay and improve fruit quality [40–44]. Ultraviolet C (UV-C) radiation is known for preventing fungal decay and enhancing phytochemical content in fruit when applied postharvest. Additionally, it has been reported that postharvest UV-C radiation induces secondary metabolites production that protect fruit against abiotic and biotic stresses [45]. Furthermore, these metabolites (phenolic compounds, anthocyanins, carotenoids) also play an important role in fruit quality with impact on human health [46]. UV-treated fruits had a lower respiration rate, higher titratable acidity and anthocyanin content, and were firmer than the untreated fruits. The percentage of free sugars increased faster in UV treated fruits at the beginning of the storage period [40]. Freshly harvested strawberries of cv. Kent, at 25–50% red were exposed to UV-C at doses of 0.25 and 1.0 kJ/m<sup>2</sup> and stored at 4 or 13°C after exposure which has resulted in controlling the decay caused by *Botrytis cinerea* at both storage temperatures and extended the shelf-life of the fruits by 4–5 days [40].

### 5.2 Chemical treatments

#### 5.2.1 Fumigation

Methyl bromide fumigation is the current treatment for postharvest strawberry disinfestation of pests such as western flower thrips [*Frankliniella occidentalis* (Pergande)] and two-spotted spider mite (*Tetranychus urticae* Koch). Due to the reduced availability and increased cost of methyl bromide (as a result of its phase out in 2005 for all uses except quarantine treatments), an alternative treatment is

desirable. Low molecular weight volatile compounds such as ethyl formate (EF) are produced by several fruits and vegetables which are important components for flavor and aroma and also have been revealed to have insecticidal and fungicidal properties [47]. Before the product reaches the market, these low molecular weight volatile compounds can potentially undergo degradation to biogenic levels in the tissues of treated commodities which is an advantage over conventional chemicals, which can persist as residues in food products.

Ethyl formate is currently in the process of being formulated with CO<sub>2</sub> for commercial use in Australia and New Zealand. Simpson et al. [48] showed that CO<sub>2</sub> in combination with Ethyl formate significantly reduced pest population (Western flower thrips and Red spider mite) without causing any damage to the fruit quality.

Nitrous oxide (NO) has been found to be ubiquitous in postharvest climacteric and nonclimacteric fruit, vegetables and flowers, with higher levels present in unripe than in ripe tissues [49, 50]. Since ethylene accumulation initiates ripening of climacteric produce and enhances senescence of nonclimacteric produce, it was speculated that application of NO might retard ripening and senescence in post-harvest tissues [51]. Strawberries are a high value fruit but marketing is limited by a short postharvest life. The postharvest life can, however, be extended by minimizing the concentration of ethylene in the atmosphere around fruit [51, 52].

Wills et al. [53] performed fumigation in strawberry in an atmosphere of anaerobic nitrogen for up to 2 h at 20°C with nitric oxide concentrations ranging from 1.0 to 4000 ml l<sup>-1</sup> then held at 20 and 5°C in air containing 0.1 ml l<sup>-1</sup> ethylene which had resulted in extension of postharvest life.

Hydrogen sulfide acts as an important gaseous regulator in plants like nitrous oxide. Fumigation with hydrogen sulfide (H<sub>2</sub>S) gas released from the H<sub>2</sub>S donor NaHS increased the postharvest shelf life of strawberry fruits depending on dose used [54]. Strawberry fruits fumigated with various doses of H<sub>2</sub>S has resulted in significantly lower rot index, maximum fruit firmness, and minimum respiration intensity and polygalacturonase activities than controls. Treatment with H<sub>2</sub>S maintained higher activity levels of enzymes catalase, ascorbate peroxidase, guaiacol peroxidase, and glutathione reductase and lowers the activities of lipoxygenase relative to untreated (controls). It also reduced hydrogen peroxide, malondialdehyde, and superoxide anion to levels below control fruits during storage. Furthermore, H<sub>2</sub>S treatment maintained higher contents of soluble proteins, reducing sugars, free amino acid, and endogenous H<sub>2</sub>S in fruits. This interprets that H<sub>2</sub>S plays an antioxidative role in enhancing postharvest shelf life of strawberry fruits [54].

### 5.2.2 *Salicylic acid*

Salicylic acid (SA) is a simple phenolic compound. It is recognized as a plant growth regulator, because of its external application effect on many plant growth physiological processes [55]. Salicylic acid (2 mM) effectively increased strawberry ascorbic acid content, fruit total antioxidant potential, total soluble solids and prevented fungal contaminations [56]. They also studied the reversible effect of SA and recommended plant SA treatment in all different growth stages like vegetative, fruit development and postharvest stage. Fruits of the plants of strawberry cv. Camarosa which received SA (0.03 mM) after 7 days at 28°C in their nutrient solution had less weight loss and decay, higher firmness and hue angle than control [57].

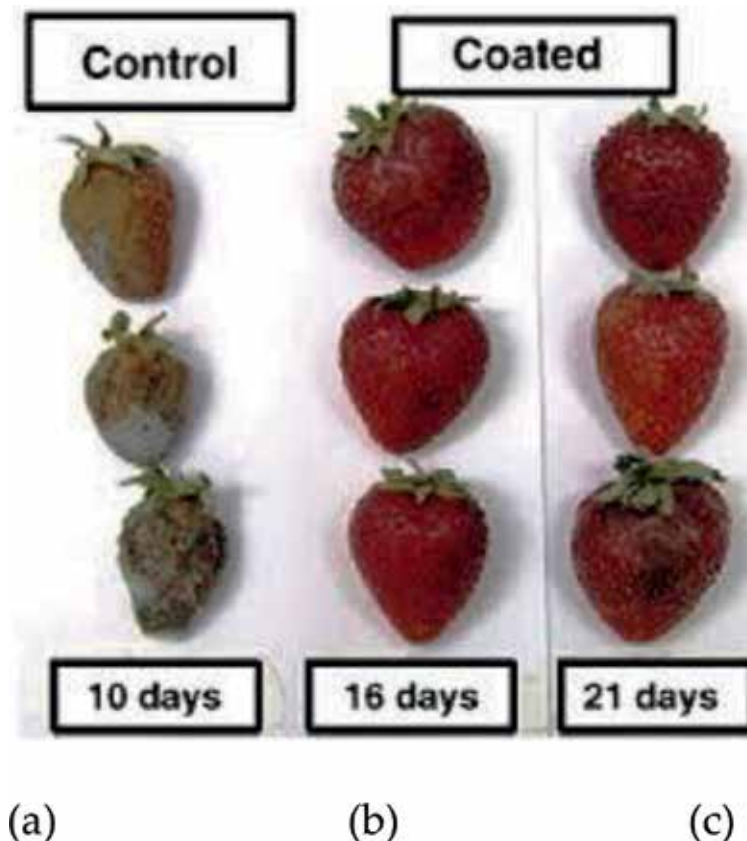
### 5.2.3 *Calcium dips*

Among secondary nutrients, calcium acts a major role in maintaining the quality of fruit and vegetables. Increasing the “Ca” content in the cell wall of fruit tissue

can aid to delay softening and mold growth and decrease the occurrence of physiological disorders [58]. Common techniques like dipping and vacuum or pressure infiltrations are used to increase cell wall Ca content of fruit tissue after harvest. The firming effect can be explained by the crosslinks formation between the carboxyl groups of polyuronide chains found in the middle lamella of the cell wall; Ca also increases cell turgor pressure [59, 60] and stabilizes the cell membrane [61]. Calcium dips have been employed to improve firmness and extend the postharvest shelf-life of a wide range of fruit and vegetables. In strawberries,  $\text{CaCl}_2$  dips in combination with heat treatment or modified atmosphere storage and refrigeration increase calcium content and fruit firmness and delay postharvest decay [62, 63]. Calcium dips were effective in decreasing surface damage and delaying both fungal decay and loss of firmness in strawberries, compared to untreated fruit [64].

#### 5.2.4 Coatings

Highly perishable fruits such as berries and tropical fruits are appropriate products to protect with coatings because they are expensive and exhibit a short storage life (**Figure 4**). Coatings can act as moisture and gas semi-permeable barriers, resulting in control of microbial growth, preservation of color and texture [64]. Strawberries, as a typical soft fruit, have a high physiological postharvest activity. As a consequence, they have short ripening and senescence periods that make marketing of this high-quality fruit a challenge.



**Figure 4.** Fruits treated with coatings have prolonged shelf life: (a) untreated fruits have 10 days of shelf life with improper color development; and (b) shelf life of fruits treated with coatings on 16th day and (c) with coatings on 21st day.

#### 5.2.4.1 Chitosan

Chitosan, a high molecular weight cationic polysaccharide, theoretically should be an ideal preservative coating material for strawberries. It has been shown to inhibit growth of several fungi [65], to induce chitinase, a defense enzyme. Due to its ability to form semi-permeable film [66], chitosan coating can be expected to modify the internal atmosphere as well as decrease the transpiration losses. Therefore a delay in ripening and control of decay by means of chitosan coating could result. Romanazzi et al. [67] found that the commercial chitosan formulation was as effective in the control of gray mold and Rhizopus rot of strawberries immersed in these solutions and kept for 4 days at  $20 \pm 1^\circ\text{C}$ .

Chitosan-based edible coatings used to extend the shelf-life and enhance the nutritional value of strawberries at either  $2^\circ\text{C}$  or 88% relative humidity (RH) for 3 weeks or  $-23^\circ\text{C}$  up to 6 months which resulted in reduced drip loss and helped to maintain textural quality of frozen strawberries after thawing. In addition, chitosan-based coatings containing calcium or Vitamin E significantly increased the content of these nutrients in both fresh and frozen fruits [68]. There was significant reduction in severity of decay and shelf-life extension on immersing strawberries stored at either  $5$  or  $10^\circ\text{C}$  in chitosan solutions of 0.5, 1.0 and 1.5 g/100 mL for 5 min at  $20^\circ\text{C}$  as compared to untreated [69]. Chitosan sprays (2, 4 and 6 g/l) significantly reduced post-harvest fungal rot and maintained the keeping quality of the fruit [70].

#### 5.2.4.2 Starch based coatings

Edible coatings can be made from food materials such as cellulose derivatives, proteins, starch, and other polysaccharides (regarded as GRAS). Starch is the most usually used agricultural raw material for biodegradable films [71]. The film-forming capacity of starches was due to presence of Amylose. Plasticizer, another important component of edible films is required to overcome film brittleness and improve extensibility and flexibility of the films. They reduce intermolecular forces and increase the movements of polymer chains. Plasticizer must be compatible with the film-forming polymer; hydrophilic compounds such as polyols (glycerol, sorbitol, polyethylene glycols) and lactic acid are frequently used in hydrophilic film formulations [72]. The effect of plasticizer on water vapor and gas permeabilities is controversial, depending on matrix, plasticizer type, and environmental conditions [73–75].

Starch-based coatings can be applied to extend storage life of strawberries (*Fragaria ananassa*) stored at  $0^\circ\text{C}$  and 84.8% relative humidity. Coatings made with starches with the higher amylose content decreased WVP and weight losses and retained fruit firmness for longer periods [76]. The coating of strawberries with cassava starch + chitosan provided the best results, with less than 6% of loss in fruit mass, lower counts of yeast and psychrophilic microorganisms and the best appearance according to the sensory analysis [77].

#### 5.2.4.3 Botanical coatings

*Aloe vera* (AV), a novel edible coating was used for fruit storage [78, 79] which has antifungal activity against several pathogenic fungi including *Botrytis cinerea* [80]. AV coatings modify the internal gas atmosphere, reduce moisture loss, softening, respiration rates, delay oxidative browning and reduce microorganism proliferation in fruits [79, 81–83]. *Aloe vera* + Ascorbic acid treatments in strawberries delayed weight loss; reduced total aerobic mesophilic, yeasts, and molds populations; and had higher SSC, vitamin C concentrations, and titratable acidity [84].

Cactus mucilage is one of the edible coating which is used for increasing shelf life of strawberries [85]. Mucilages are generally hetero-polysaccharides obtained from plant stems [86]. They may find applications in cosmetics, food, pharmaceutical and other industries. The complex polysaccharide is a part of dietary fiber and has the capacity to absorb more amounts of water by dissolving and dispersing itself and forming gelatinous or viscous colloids [87]. Cactus mucilage as a coating is its low cost.

### **5.3 Storage**

#### *5.3.1 Modified-atmosphere packaging (MAP)*

Modified-atmosphere packaging (MAP) of fruit and vegetables is becoming a popular method of extending shelf life [88]. Strawberries fumigated with acetic acid at 5.4 mg/L followed by modified atmosphere packing were found to be free of decay compared to 89% rotted for the control fruit stored for 14 days at 5°C [89].

#### *5.3.2 Controlled atmosphere storage*

The use of a carbon dioxide enriched atmosphere is an extensively used postharvest practice to manage and control fungal decay in freshly harvested fruits and vegetable products. Numerous studies have revealed that controlled atmosphere storage of different cultivated strawberry varieties may enhance their shelf life by slowing down both fungal decay and senescence. These effects are linked with the reduction of respiration and ethylene production rates [51, 90, 91]. Exposure of fruits to high levels of CO<sub>2</sub> during cold storage showed enhancement of firmness [92, 93] and resistant to decay [51]. However, combinations of high CO<sub>2</sub> and low O<sub>2</sub> atmospheres improve most strawberry quality traits, increase in generation of off-flavor compounds like ethanol and ethyl acetate, producing an adverse sensory effect [94]. The atmosphere of high CO<sub>2</sub> and high O<sub>2</sub> do not ease these off-flavor problems and show to persuade a synergistic effect that even increases the assembly of fermentative metabolites [94, 95]. Allan and Hadwiger [96] studied that 10% CO<sub>2</sub>/11% O<sub>2</sub> combination had efficiently prolonged the shelf life of wild strawberries by maintaining the quality parameters within acceptable values, through inhibiting the development of *Botrytis cinerea*, without significantly modifying consumer acceptance.

### **5.4 Packaging and transport**

In order to avoid deterioration during storage, strawberries need to be well packed immediately after harvest. Cold-chain system is regularly used to conserve the quality and flavor for a prolonged period.

#### *5.4.1 Cold-chain system*

Precooled strawberries are stored at a low temperature in a cold store, or transported for marketing in a refrigerated van. This system enables the fruits to remain fresh until they reach the consumers. In a well-organized cold-chain system, cold air should be well dispersed within the boxes used for packaging. Furthermore, boxes should not lose their shape even if they become moist. Each box should hold the correct amount of fruit, and be of an appropriate size to reduce the cost of distribution.

#### *5.4.2 Packaging films*

Packaging of fruits with polymeric films is often used to prevent moisture loss, to protect against mechanical damage, and to achieve a better appearance [97].

Packaging strawberries with plastic films immediately after harvest is only technique to prevent water loss during storage. The water loss may lead to shriveling and a dull appearance of the epidermis having a negative effect on the appearance of the fruit.

Different types of packaging films include:

Perforated cellophane sheets (CS): these were placed on top of the baskets and fixed with elastic bands.

Low density polyethylene bags (PB): these were heat-sealed after introducing one or more baskets per bag.

Polyvinyl chloride films (PVC): PVC films has resulted in better fruit weight and firmness retention of fruits of strawberry especially in the last 7 days of storage [98].

The use of Low density polyethylene bags as packing films in strawberry has resulted in the lowest weight losses, conductivity and degree of fruit decay, together with the highest firmness values [99].

#### 5.4.3 Hydrophilic starch films

They offer good barrier to oxygen and carbon dioxide transmission but a poor barrier to water vapor under certain conditions of relative humidity (RH) and temperature [97, 100]. These characteristics are favorable for preservation of quality of fruits and vegetables, since they lead to a decline in respiration rate by restricting the exposure to ambient O<sub>2</sub> and increasing internal CO<sub>2</sub>, thus delaying ripening. The poor water vapor barrier allows mobility of water across the film, thus preventing water condensation that can be a possible source of microbial decay in soft fruits and vegetables [101].

#### 5.4.4 Corrugated fiber board boxes

This improved method of packaging consists of an outer box made of corrugated fibreboard (**Figure 5**), having a capacity of 6 kg, and 6 inner boxes made of cardboard, each box have a holding capability of 1 kg fruit. The external box is 54 cm long × 36 cm wide × 9 cm high, and was designed to occupy 96% of the base area.

#### 5.4.5 Ventilation holes

In order to facilitate the movement of cold air, ventilation holes (**Figure 6(a)** and **(b)**) were made on the sides of the box and the internal partitions. Ventilation



**Figure 5.**  
*Packaging of strawberries: (a) plastic punnets (b) Corrugated fiber board box.*



**Figure 6.** (a) Strawberries packed in corrugated fiber board boxes (side view) and (b) strawberries packed in corrugated fiber board boxes with laminated polyethylene (top view).

holes to surface area ratio in the outer box are 4.5 per cent, whereas, that of the internal partitions is 10.5 per cent. The passage of cold air through the holes shortens the precooling time and makes more uniform cooling.

In addition, the ventilation holes are circular and measures less than 20 mm in diameter, which are small enough to avoid the strawberries from being caught in the holes. The box is a folding type to prevent shape distortion, which repeatedly happens to other packaging boxes as a result of dampness. With this novel box, the strawberries can be securely stored for a longer time.

## 6. Conclusion

Strawberry, which possesses attractive color, palpable taste and significant mineral and vitamin content, is highly perishable. In the present trend where consumers want food to be of medicinal value, strawberry is the promising one. By following appropriate postharvest practices growers can minimize losses and preserve the fruit in fresh form with good quality for longer duration. Further, there are tremendous prospects of commercial utilization of strawberry for extraction of natural color and have great potential as raw material for production of diverse value added processed product and thereby develop agro-industry. Strawberry can be regarded as the fruit crop of future.



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# Physical, Chemical and Processing Postharvest Technologies in Strawberry

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## Abstract

Strawberry (*Fragaria × ananassa*) is a fruit of great acceptance worldwide but has characteristics that make it a highly perishable fruit, with shelf life of about a week, which makes it difficult to transport and store it to consumer places. Throughout the years, post-harvest techniques have been studied to extend their useful life and improve their properties. Strawberry deterioration may be due to various factors such as overripe, fungal involvement, moisture loss, mechanical damage, among others. Among the techniques which have been tried to slow the deterioration of the fruit are the use of modified atmospheres and treatments gases, use of edible coatings and smart packings, application of radiation of various types, use of chemical treatments among many others. In this chapter, we will examine the most relevant treatments applied to the strawberry to extend its useful life and improve its organoleptic quality that have been reported in the literature.

**Keywords:** modified atmosphere, radiation, quality, physiology, shelf life

## 1. Introduction

Strawberry (*Fragaria × ananassa*) is considered a non-climacteric fruit [1] that is, it does not continue its maturation process after being cultivated. Coming from the *Rosaceae* family, it is cultivated in various countries around the world and consumed throughout the world due to its taste, smell and color. It is consumed fresh, dry, in preserves and culinary preparations, its transport can be carried out in fresh or in freezing, which can alter its organoleptic characteristics.

Being a non-climacteric fruit, it should be grown at its peak ripeness; however, this makes shorter lifespan compared to climacteric fruits, which can be matured along transportation. The high moisture content of the fruit and the characteristics of its skin make it susceptible to mechanical damage and the proliferation of fungi and other microorganisms that damage the fruit.

For the realization of this chapter, more than 100 scientific articles from different databases were searched using search parameters “strawberry” “postharvest” “shelf life”. As can be seen in **Figure 1**, most studies have focused on the use of treatments with gases and modified atmospheres to extend the useful life of the product while maintaining its quality parameters with about 21.3% of the total of studies



**Figure 1.**  
Postharvest treatments used in strawberry.

reviewed (violet zone). Second, the use of physical elicitors such as radiation, ultrasound, changes in pressure among others, to reduce the biological load on the surface of the fruits, activate defense mechanisms of plant tissue or the generation of compounds to maintain the shelf life of fruits, covering just under 15% of those surveyed items (blue zone).

Other technologies in postharvest have been applied in strawberry as thermal treatments, application of edible coatings, use of chemical solutions in fruits or the application of several technologies at the same time to generate synergistic responses in the product.

Each of the technologies studied has its advantages and disadvantages, as well as its application in various scenarios for the transport and storage of the product. The use of each one depends on the amount of fruit to be treated, as well as the cost of application, the need on the part of the producers and the demands on the part of the buyers.

## 2. Modified atmospheres and gas application

The use of modified atmospheres, controlled atmospheres and application of gases in post-harvest is one of the treatments with greater acceptance in the post-harvest industry [2]. The use of these gases has an impact on the appearance and texture of the fruits; however, the effects on taste and odor are not yet clear and may differ from product to product. In the studies carried out, it has been found that the use of modified atmospheres generates changes in post-harvest parameters such as titratable acidity (TA), total soluble solids (TSS), sugars and organic acids and metabolites derived from fermentative processes.

The use of carbon dioxide as part of a modified atmosphere has shown positive effects in preserving the sensory characteristics of strawberry [3, 4]. Studies on the effect of the application of carbon dioxide have shown that it generates stress in the tissue of the fruit generating an increase in the  $\gamma$ -aminobutyric acid (GABA), which, in intermediate levels, activates mechanisms that allow the fruit to maintain the color and texture suitable for consumption [5].

Short exposure to high levels of carbon dioxide has shown that it is able to reduce the chemical and physical phenomena associated with deterioration of the fruit, decreasing tissue ATP levels and generating a low ethanol metabolism, unlike when stored in the presence of air, which generates an increase in the ATP and an explosion of the processes of fermentation in the tissue, leading to its putrefaction [6].

The use of carbon dioxide also has an effect on the proliferation of microorganisms such as *Botrytis cinerea*, which is responsible for the loss of strawberry quality. The studies showed that use of concentrations between 5% and 10% of CO<sub>2</sub> helps to reduce the proliferation of Botrytis, without generating negative impacts on parameters such as TSS and TA, in addition to maintaining a uniform and attractive color for the consumer [7, 8].

The application of 1-methylcyclopropene (1-MCP) has been an alternative studied to manage the deterioration process in strawberry. Several studies have been applied dose of the gas to decrease the rate of senescence, with positive results without affecting quality in doses from 0.5  $\mu\text{L L}^{-1}$  to 5  $\mu\text{L L}^{-1}$ , however, at higher doses, the effects of deterioration accelerated [9–13].

The management of the production and/or presence of ethylene in the packing atmosphere or in post-harvest treatment is one of the most used techniques for managing the ripening speed in fruits and vegetables, sometimes, it is desirable to decrease the presence of this gas, but supplementation is also used to improve the post-harvest quality of various agricultural products. In the case of strawberry, some studies have been conducted in this direction and in search of the elucidation of the biochemical processes responsible for the response of the tissue against this gas [14–16].

The use of atmospheres saturated with oxygen has been studied with mixed results. At high concentrations of O<sub>2</sub> the rate of deterioration was lower [17] but studies on the release of volatile compounds from the treated fruits showed that the application of supplemental oxygen stress generated in the metabolism leading to the production of compounds related to alcoholic fermentation, raising questions about the effectiveness of such treatment [18, 19]. On the other hand, the use of ozone (O<sub>3</sub>) as a treatment in strawberry showed dissimilar results in the control of the proliferation of pathogens [20, 21], but the use of water enriched with ozone as a cleaning method proved to reduce the biological load on the surface of the fruit without affecting its turgidity or firmness [22]. Another approach to the use of oxygen compounds for post-harvest treatment is the in-situ generation of reactive oxygen species (ROS), which showed positive effects in variables measured as TSS, acidity, maturity stage, among others. [23].

Among the studies analyzed, one stands out where nitrous oxide was used as a regulating agent for the growth of fungi and molds, with positive results [24]. In the aspect of modified atmospheres, packages have been developed that directly regulate the concentrations of different gases throughout the transport and storage of the product, managing to extend the useful life of strawberry in about 10 days in comparison to standard packages [25, 26].

### 3. Physical elicitors

The use of postharvest physical elicitors has been studied for several decades. This type of technology has the advantage of low operating costs and the rapid

amortization of the initial investment, in addition to generating positive responses such as the generation of metabolites of interest in the treated products, but its main disadvantage is the time for standardization of the process and the variability of the generated responses that depend on the matrix subjected to the stimulation.

Most of the studies consulted that applied a physical elicitor used ultraviolet (UV) radiation with special emphasis on ultraviolet C radiation (UV-C), which has a higher energy than A and B radiation. Different studies on the use of radiation UV-C have demonstrated their ability to decrease the biological load of the fruit without affecting sensory properties such as color, firmness, texture, humidity, among others. [27–30].

Studies on the impact of the application of ultraviolet C radiation on phytochemical processes in tissue products have shown that it has a direct impact on the synthetic route of phenylpropanoids and phenylalanine ammonia-lyase (PAL) [31–34], which it has effects on the production of secondary metabolites such as polyphenols, anthocyanins and oxygenates, that have been identified as families of compounds of interest for their antioxidant activity, increasing the benefits of strawberry intake for the final consumer [35, 36].

The application of ultraviolet B radiation (UV-B) showed similar effects as those found with UV-C, but the exposure and dose times are higher to achieve the same results. This increase in time and dose generates an increase in the cost of its application as a post-harvest treatment, but UV-B radiation is more secure at the genetic level, since its impact is lower in the DNA chains, decreasing the possibility of death cellular or generation of mutations in the tissue, and in turn, is less dangerous to the operator than UV-C radiation [37].

The use of pulsed light (PL) as post-harvest treatment was investigated by Duarte-Molina et al. Finding that it has positive effects on the texture and firmness of the product compared to the control samples, diminishing the effect of pathogens and longer shelf life without negative effects on other postharvest parameters, turning this technology into a promising alternative in the handling of strawberry [38].

Gamma radiation used as a process of sanitization in food and post-harvest treatments was booming in the last decades of the twentieth century [39]. However, the consumer's fear of the presence of residual radiation in the products led to the labeling of these products as subject to gamma rays and the subsequent rejection of these by consumers. But its use as a means of sanitization with low effect on the texture of the product has been tested in strawberry with positive effects at low levels of radiation [40].

Another of the alternatives for handling the biological load naturally present in strawberry is its exposure to low pressures in, obtaining positive effects at 0.25 atm per 24 hours [41]. These treatments at reduced pressures also showed effects on the antioxidant capacity, which suggests a positive effect on the stimulation of various metabolic pathways such as those mentioned above [42].

Using the response surface methodology (RSM) parameters were optimized for the use of ultrasound as post-harvest treatment in strawberry, finding an optimum in the power parameters and exposure time to 250 kw and 9.8 minutes respectively [43], decreasing the incidence of fungi and molds without affecting the quality of the product.

#### **4. Thermal treatments**

The thermal treatments used in postharvest seek to eliminate the biological load that is in the skin of the products through the application of heat or cold for a certain period. In the case of heating, the temperature used must be high enough to eliminate fungal spores and mold, but not so much to generate changes in the fruit

such as Maillard reactions, caramelization or oxidation. The time of exposure to these temperatures is also critical. In the case of treatments based on the application of cold, the temperature should not be so low to generate tissue damage by freezing the water inside the cells, but enough to inactivate the biological load.

In the case of the application of heat, this can be done through the immersion of the strawberry in water, as was done in [44]. Four temperatures (25, 35, 45 and 55°C) were tested for 15 minutes. The best results were obtained at 45°C, where the lowest losses were obtained in comparison to the other treatments and to the control, however, the color of the strawberry was affected in a negative way, which was confirmed by another study [45].

Other studies used hot air at temperatures between 35 and 55°C in forced air ovens, with an exposure time between 1 and 5 hours. Subsequent analyses showed that hot air treatment had a positive impact on strawberry shelf-time, and on parameters such as firmness, respiratory rate, anthocyanin content, titratable acidity and TSS [46–50], however, the loss of color measured through a colorimeter was also observed in this technique. Further investigation determined that the use of hot air affects the expression of several genes in strawberry (FaPG1, FaPLB, FaPLC, FaAra1, FaβGal4) and the greater stress load is evident in the cell wall, which generates an increase in the amount of cellulose, hemicellulose, and lignin in fruits, which in turn explains the preservation of cell structure, which decreases the incidence of *Botrytis cinerea* [51].

An alternative to the use of hot air for heating strawberry is the use of Far Infrared Radiation (FIR), which provides the possibility of uniform heating on the surface [48]. Simulations carried out using the Monte Carlo method and validated through a thermal imaging camera showed that an optimal control over the surface temperature in strawberry can be achieved below the critical limit of 50°C along with a uniform heating, which would be maintained the post-harvest quality and the shelf life would be lengthened. The use of low temperatures for the preservation of strawberry has been studied as a traditional alternative for the preservation of shelf life in long periods of storage, however, the temperature used for cooling, as well as the cooling rate are decisive factors on product quality. A first approach is to use temperatures above 0°C that will reduce the natural biological processes of both the fruit and biological contaminants. In the study carried out by Ayala-Zavala and others [52] temperatures of 0, 5 and 10°C were tested, finding that parameters such as antioxidant capacity and the profile of volatile compounds were better at temperatures above 0°C.

Under more extreme conditions, strawberry was stored at temperatures of –40°C for 6 months to subsequently measure parameters such as reducing sugars, total phenols, color, antioxidant capacity, brightness and firmness of the skin of the fruit [53]. It was found that storage at that temperature maintains the chemical and physical characteristics of the fruits, finding only difference between the cultivation techniques used, which was part of the reported research.

As mentioned above, the cooling rate is a critical parameter when performing cold treatments. If a fruit cools or freezes quickly, prevents large ice crystals form inside the cells, which could cause damage to the cell wall, decreasing the quality of the product and increasing the possibility of infection by pathogens. The simulation of cooling systems for strawberry packaging has been studied in order to define the optimal parameters of air speed and temperature of the same to achieve a uniform and fast cooling [54].

## **5. Chemical treatments**

The use of chemical substances to promote or delay the maturation and senescence processes in fruits and vegetables has been widely studied. They have been

used from inorganic salts to a wide variety of organic compounds that have been shown to have an impact on the metabolism of plant tissue. Generally, the application of said compounds is carried out by immersing the product in a solution of the compound or by spraying it. The compounds used must be safe for human consumption and their concentration must not alter the organoleptic properties of the treated product.

To facilitate the review of the articles, it is convenient to divide the chemical treatments into three large areas; inorganic compounds, organic compounds, and essential oils. Essential oils have become very important in post-harvest processing, as well as in industries such as food and cosmetics. It has been found that essential oils have different properties ranging from antioxidant capacity to inhibit the proliferation of fungi, bacteria and viruses.

Calcium chloride ( $\text{CaCl}_2$ ) is one of the most used inorganic compounds in post-harvest treatment in various products in concentrations between 1–4%. In the studies consulted, positive results were obtained by stopping the deterioration process in strawberry, maintaining the parameters of sensory quality [55–57].

Hydrogen sulfide ( $\text{H}_2\text{S}$ ) is a compound that plays a vital role in the metabolism of the maturation and senescence of the fruits. The supplementation of this compound through the fumigation of the fruits prolongs the useful life of the product directly depending on the dose used. It was also identified that hydrogen sulfide maintains the activity of families of enzymes such as catalase, guaiacol peroxidase, ascorbate peroxidase, and glutathione reductase [58].

Another possible use of inorganic compounds in postharvest is the sanitization of products. A mixture of peracetic acid (PAA) and hydrogen peroxide is nebulized in strawberry samples in concentrations ranging from 3.4 to 116  $\mu\text{L PAA L}^{-1}$  air chamber. The quantification of the concentration of phenolic compounds showed degradation of this class of compounds at certain concentrations of PAA, being the anthocyanins the most affected, followed by the proanthocyanidins with low level of polymerization and hydroxycinnamic acid derivatives [59].

The addition of organic compounds in solution or through their vaporization in the post-harvest stage or packaging has various mechanisms of action to preserve the quality of the products. One of the most studied compounds in strawberry pure methyl jasmonate (MJ) or in solution with ethanol [60]. The different studies concluded that the use of MJ in strawberry increases the concentration of volatile compounds such as Methyl acetate, isoamyl acetate, ethyl hexanoate, butyl acetate, and hexyl acetate. Also, the useful life increased in comparison to those that were not submitted to the treatment, as well as the antioxidant capacity of the fruit [61, 62]. Similar results were obtained when using 2-nonanone in strawberry systematically released by packaging and tested under shelf conditions [63].

In floriculture the use of salicylic acid as a preservative in flowers and foliage is very common, studies have also been carried out on the possibility of its use as an agent that modulates the release of ethylene in fruits such as strawberry. The effect of salicylic acid is independent of the concentration and has as an additional advantage its ability to control the proliferation of fungi, extending the shell life [64–66].

The gibberellic acid (GA3) is a maturation retarder commonly used in postharvest and its application in strawberry has been investigated. Using partially mature samples, the application of gibberellic acid delays the process of color generation in fruits, together with the activity of PAL and other enzymes such as chlorophyllase and peroxidase, decreasing the speed of fruit ripening [67].

Apart from the aforementioned compounds studies have been conducted with Ethyl pyruvate [68], melatonin [69, 70] and acetic acid from baby corn [71] with

very promising results, however, the high specialization of these compounds, as well as the necessary infrastructure for their application in post-harvest, make it difficult to implement treatments based on these results for small and medium producers of strawberry.

Essential oils are a mixture of a large number of organic compounds of the family of volatile terpenoids (monoterpenes, sesquiterpenes), mixed with other compounds such as aldehydes, ketones, esters, ethers among others. The amount and which compounds are present in an essential oil depend directly on the source from which it is extracted and on the extraction methodology. Essential oils are obtained through steam distillation, by cold pressure extraction and dissolution in vegetable oils.

In the case of strawberry, tea tree oil (TTO) has been tested as an antifungal agent, obtaining positive results in the decrease of the proliferation of *Botrytis cinerea* and *Rhizopus stolonifer* in strawberry, which are mainly responsible for the damage by pathogens in postharvest [72]. This same essential oil was tested as a pre-harvest treatment in strawberry, also obtaining a decrease in the impact of fungi on the fruits, although positive effects were also evidenced in parameters such as the firmness, color and quantity of polyphenols [73].

Essential oils are alternatives generally considered safe against the use of conventional chemicals in products for human consumption. The use of essential oil of *Satureja* species (*S. hortensis*, *S. spicigera*, and *S. khuzistanica*) as a fungicidal agent has been investigated. The essential oils were characterized using gas chromatography coupled to mass spectrometry, determining that the major compounds were carvacrol, thymol,  $\gamma$ -terpinene and p-cymene [74]. The essential oils tested showed ability to inhibit the growth of *Penicillium digitatum*, *Botrytis cinerea* and *Rhizopus stolonifer* in strawberry under storage conditions.

## 6. Edible coatings

Edible packaging has become one of the most booming research topics in recent decades in food and post-harvest. An edible package must have characteristics such as generating a uniform coating on the surface of the fruit, allowing and/or regulating the rate of respiration of the fruit to maintain the sensory quality thereof, be inert and harmless to the human being, be easily applicable and fast dry. An edible package can be applied either through immersion in the coating solution or by sprinkling using air under pressure.

Chitosan is one of the most commonly used coatings at industrial level thanks to its null toxicity and generation of a semipermeable membrane that allows the passage of moisture and gases, preventing the start of anaerobic fermentation processes [75].

In studies conducted in strawberry, the application of chitosan decreased the proliferation of fungi and molds that affect the quality of the fruit [1, 76, 77]. The use of additives such as glycerol, olive oil, extracts of essential oils, among others, have been studied to improve certain qualities of chitosan, such as tensile strength, gas exchange capacity, antifungal and antibacterial capacity, among others. [78–81]. The study of the application of nanocomposites based on titanium and other elements have proven to provide functional properties to the coatings, from being only a coating to extend the useful life to provide functional food properties to the products in which they are used [81–84].

But not only has chitosan been tested in strawberry, other substrates and substances have been tested in search of economic and technical alternatives to traditional methods. The substances used range from coatings based on gluten, methylcellulose, quinoa protein, *Aloe vera*, silk fibroin, a mixture of various polysaccharides and arabic gum [85–92].

## 7. Combined treatments

Sometimes, the application of two or more post-harvest treatments generates a synergistic effect on the quality and maintenance of the product's useful life. The order in which the treatments are applied determines the effectiveness of the final result [93].

Immersion of strawberry in calcium gluconate subsequently be coated with a formulation of 1% chitosan or chitosan-sodium gluconate was assayed by Hernández-Munoz; better results are obtained when using the formulation of both components [94]. The use of a mixture of chitosan together with organic acids, calcium and vegetable extracts demonstrated a positive impact on fungal control in pre-harvest and post-harvest [95, 96] and the use of an edible coating based on a mixture of *Aloe vera* and beeswax, coupled with the control of temperature and humidity in storage, decreased the percentage of post-harvest losses in strawberry as reported by Affan [97].

On the other hand, the use of physical elicitors in combination of controlled atmospheres has been shown to generate an additive effect in the conservation of strawberry quality. The combination of ozone, atmospheres with high concentrations of oxygen and carbon dioxide together with the application of UV-C proved to extend the useful life as well as increase the content of polyphenols and ascorbic acid present in the fruit [98]. Likewise, the application of chlorine dioxide, fumaric acid linked to UV-C, decreased the biological load on the fruits [99]. Other compounds or treatments used in combination with UV-C radiation are hot water and salicylic acid [100–102].

Other combined treatments include the use of nitric oxide, ethylene and low temperatures [103], low density polyethylene with nanoparticles of titanium oxide to actively control the respiration of the fruit [104], Use of specific light intensities after washing the fruits in chlorine solution [105] and the use of nitrogen for strawberry freezing at  $-20^{\circ}\text{C}$  after sanitization with 50 ppm chlorine [106].

## 8. Conclusions

The strawberry is a fruit of worldwide interest for its sensory properties and nutritional quality, but its physical and chemical characteristics generate problems in storage and transport. In order to face these challenges, several post-harvest techniques have been tried to know the impact on the quality and characteristics of the fruit. Each technique has advantages and disadvantages and the implementation of one or more of these post-harvest techniques will depend on economic, technical and social factors of the growing region.

In the case of strawberry, the most used techniques are those associated with modified atmospheres, since they allow to regulate the process of senescence of the fruit, but have the disadvantage that, if this atmosphere is altered, the quality of the product will be altered. Physical elicitors have also been widely studied with positive results.

## Conflict of interest

There is no conflict of interest on the part of the authors.



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# Postharvest Quality Management of Strawberries

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## Abstract

Strawberry fruit (*Fragaria × ananassa*), a genus of the Rosaceae family, is the most commonly consumed berry fruit crop worldwide and is valued for its unique flavor and nutritional quality. Strawberries are expensive and filled with vitamins, fiber, and antioxidants. The susceptibility of strawberry fruit to postharvest diseases and decline of quality attributes increases after harvest and through extended storage, and as a consequence changes in physiological and biochemical parameters. Exogenous spraying, coating, or dipping was widely used to prolong the shelf life of strawberries. The temperature, atmospheric gas, and exogenous postharvest treatment (spraying, coating, or dipping) contribute to the maintenance of the fruit's postharvest quality. Previous studies examined the effects of exogenous treatments on strawberry quality. In this review, we will thus discuss the influence of postharvest treatment on strawberry postharvest shelf life and quality management during storage conditions.

**Keywords:** strawberry fruit, edible and essential oil coatings, exogenous chemicals, control atmosphere, physiological and biochemical changes, quality and shelf life

## 1. Introduction

Strawberry (*Fragaria × ananassa* Duch.) is perennial herb plant that belongs to family *Rosaceae* and genus *Fragaria*. Most of the members in genus *Fragaria* are characterized by polyploidy and their cultivation in temperate zones of the world. In addition to this, other members of *Rosaceae* family (such as apple, plum, pear, and others) are climacteric in nature; however, strawberry is nonclimacteric since fruit maturation and ripening is almost independent from ethylene biosynthesis. The modern cultivated strawberry fruit is octoploid hybrid (8n) in genetic makeup, containing 56 chromosomes. This hybrid was developed by successful cross between Chilean strawberry (*Fragaria chiloensis*) and meadow strawberry (*Fragaria virginiana*) in 1780 in USA.

Botanically, fruit is not berry but it is aggregate accessory since fleshy part is derived from central receptacle that holds floral ovary. The outermost fruit surface contains imbedded achene (average 200 on each strawberry) that encompasses seeds inside. Strawberry is one of the most adorable fruit crop often characterized by its unique organoleptic properties and nutraceutical importance. Fresh slices of strawberry fruit are rich source of flavonoids, fibers, vitamins, potassium, and diverse array of phenolic acids such as hydroxycinnamic and hydroxybenzoic acids [1].

Strawberry, although nonclimacteric, is one of the most perishable fruit. During ripening, various physiological, morphological, and compositional changes transform inedible strawberry fruit into a highly cherished fruit. Loss of chlorophyll, gain of anthocyanin, increase in sugars, ascorbic acid and pectin, and reduction in acidity, phenolic and cellulose occur during the ripening stage. Also, fruit softening due to disassembly of cell wall mainly due to dissolution of middle lamellae occurs during this phase. Finally, fruits are harvested at fully ripened stage for its markedly favorable organoleptic features. However, these desirable fruit characters are accompanied by high respiration and tissue softening rate, water loss and susceptibility to physical damage and, hence, fungus infestations, particularly *Botrytis rot* and *Rhizopus rot*. Therefore, it is of utmost importance to develop and strictly adhere to strawberry-specific postharvest management procedures to ensure fruit quality and quantity for longer period.

The postharvest practices are aimed at slowing the respiration rate and water loss, maintaining fruit firmness and minimizing the growth of pathogens. Strawberry fruits have a narrow marketable window of 7–10 days if special care is taken. The objective of this chapter is to compile and comprehensively describe the general code of practices which should be adopted during harvest and postharvest operations of strawberry to reduce the losses and consequently resolving the quality management issues.

### 1.1 Uses, nutritional value, and health benefits

Fresh fruit of strawberry is rich source of magnesium and potassium which help smooth circulation of blood pressure and relaxation of nerves. Several reports have shown that vitamin C is more abundant in strawberry than in other fruits including citrus. Strawberry also contains niacin that is well renowned for its positive effects against cardiovascular diseases.

Strawberry contains high content of anthocyanins, flavonols (myricetin and quercetin derivatives), flavanols (catechin, epicatechin, proanthocyanidin B1 and B2), dihydrochalcones (phloridzin) [1, 2]. Fisetin (7,3',4'-flavon-3-ol) is another novel flavonol biosynthesized in the strawberry fruit through branchy and intricate phenyl-propanoid pathway. Fisetin plays major role for improving antioxidant activity and anticancer ability by blocking PI3K/AKT/mTOR pathway [3]. The fleshy fruit of strawberry contains significant quantity of hydrolysable tannins (ellagitannins and gallotannins). The estimated content of ellagitannin ranges 8–23 mg per 100 g fresh weight of strawberry fruit. After ingestion of ellagitannins, they reach in stomach, followed by small intestine and finally in colon where they are catalyzed into urolithins by gut bacteria [4].

Deep red coloration of strawberry fruit is bestowed by pelargonidin-3-glucoside and cyanidin-3-glucoside (anthocyanin). Besides this, four minor pigments with purple shade anthocyanin have also been reported such as (1) epicatechin (4 $\alpha$  → 8) pelargonidin 3-O- $\beta$ -glucopyranoside, (2) catechin (4 $\alpha$  → 8) pelargonidin 3-O- $\beta$ -glucopyranoside, (3) epiafzelechin (4 $\alpha$  → 8) pelargonidin 3-O- $\beta$ -glucopyranoside, and (4) afzelechin (4 $\alpha$  → 8) pelargonidin 3-O- $\beta$ -glucopyranoside [5, 6]. Anthocyanin contributes nearly 75% of total polyphenols in the strawberry fruits. The total anthocyanin content ranges 20–60 mg 100<sup>-1</sup> g fresh weight of strawberry fruit that is rapidly absorbed in the body after catalytic activity of phase II enzymes in small intestine or liver.

### 1.2 World production

Poland has the largest area under strawberry cultivation; however, due to utilization of technological advances and advantage of climatic conditions, USA is the

largest producer of strawberry fruit by producing more than 1,420,570 tons from 21,242 hectares during 2016 (**Figure 1**). California produces 88% of total strawberry fruit produced in USA with cultivation area 35,915 acre, generating \$316,394,000 worth revenue by exporting to Japan, Canada, and other European countries [7].

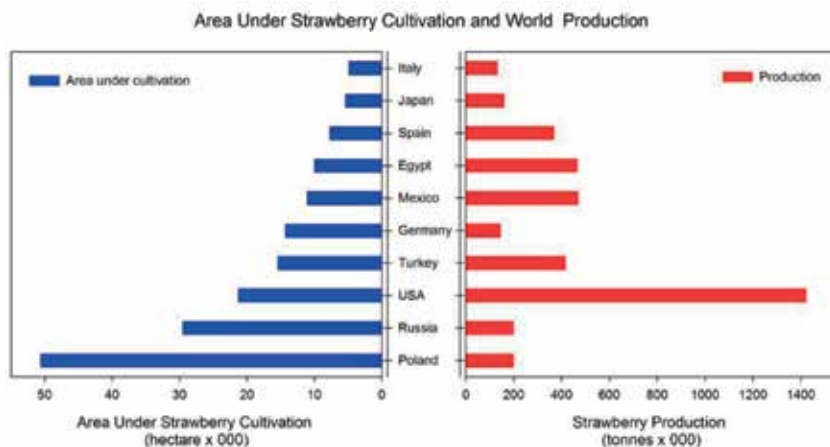
In USA, it is mainly grown along the coastal belt of California followed by Florida, Oregon, North Carolina, Michigan, and Washington. However, temperate climate of California allows the year-round growing of strawberry thus producing more strawberry per hectare than any other state [8]. Whereas the climatic condition of other states limits the cultivation of strawberry for only 5 months.

Followed by USA, Mexico, Egypt, Turkey, Spain, Russia, and Poland are the major producers in the world. Major producing areas in Mexico include Guanajuato, Michoacán, Jalisco (Central Mexico), and Baja California. During last decade (2004–2014), exported volume of strawberries in Mexico augmented nearly four times to that of 2003 [9].

### 1.3 Cultivation

Strawberry can be cultivated on wide range of soil types from sandy to clay loam. Well-drained sandy-loam soil of pH (5.5–6.5), enriched with organic matter and sufficient water-holding capacity is excellent for plant growth. Alkaline (pH > 8.5) or heavy clayey soils with poor drainage are not suitable for planting strawberry and affect plant growth by encouraging disease development. Strawberry plants are very sensitive to saline soil. The root system of strawberry plant is shallow; that is, 20–30 primary active roots are present in the upper 30–40 cm layer of well-drained soil. Depending on soil condition, fertilizer with an NPK ratio at 1–2–1 or 1–3–1 should be applied at the time of soil preparation. Planting strawberry along with application of perlite, peat-moss, or other organic media enhances the plant growth.

Commercially, there are three different methods used for cultivation of strawberry plants. The most common planting method is matted-row-system. Strawberry plants are cultivated at 45–60 cm plant-to-plant distance on raised beds and runners have sufficient space to grow from the mother plants. This method is cheap and requires least labor for agronomic practices. Spaced-row system is the second type of strawberry planting system. Plants are cultivated on narrow ridges (20 cm high) at the same distance (45–60 cm); however, runners are less likely to



**Figure 1.**  
*Strawberry area and production in the world.*

grow further. Runners are pruned after growth length reaches up to 12 cm. Though it is labor intensive method, it produces high-quality fruit with large berry size and less disease incidence. Third planting system is often suitable for the hilly areas. The runners are completely removed from the mother plant to yield high and better quality. This method is also labor intensive. After removal of runners, mother plants flourish quickly and produce high yield.

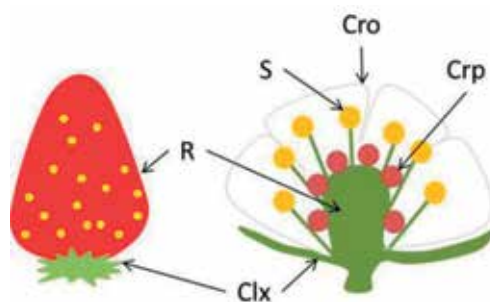
After soil preparation, organic mulch (dry straw) or plastic sheet is spread over the ridges leaving plant cultivation holes in aisles. It helps to maintain the soil temperature, moisture content, and suppress the germination of weeds or other unwanted plants. Soon after transplantation of the suckers, water is applied through drip irrigation or other available source. Water logging due to frequent irrigation or too late watering affects plant growth, consequently berry yield. Sprinkler irrigation system has also gained popularity due to water saving and being cheap installation. Low doses of fertilizer can also be applied by drip or sprinkler (foliar application) irrigation system (fertigation).

#### 1.4 Fruit growth, development, and maturation

Strawberry cultivars can be classified on the basis of their bearing habit as June-bearing, ever bearing and day-neutral cultivars. “June-bearing” strawberry cultivars produce flower when day-length is short (<12 h). And “ever-bearing” cultivars bloom only when day light is available for relatively long durations (>12 h). Similarly, day-neutral cultivars are independent from day-length but flower only when the temperature range is 18–24°C. Hence, choice of cultivars depends on geographical location, yield potential, marketability, and disease susceptibility [10].

Generally, strawberry flower is hermaphrodite having white petals with 22–25 yellow-colored anthers and central disk of stigma placed over receptacle. Although flowers are self-fertile, viability of stigma surpasses the duration of pollen liberation from anthers that increases the chances of cross-pollination. The basal end of the stamen contains nectar that helps to recruit the pollinator. Usually, stigma receptivity lasts from 7 to 10 days, while pollens are available during 1–3 days. Flowers are produced in clusters and the earliest flower sets first fruit with largest size due to having more number of ovules (**Figure 2**).

Poor pollination results in reduced number of fruit set. Sometimes, small and misshaped fruits are produced due to poor pollination. Hence, honey bee cages (approximately, one large bee hive for 1 acre) are recommended for commercial cultivation of strawberry that can increase fruit set (up to 10%) and significantly reduced share of deformed berries.



**Figure 2.** Morphological representation of flower and fruit parts. S, stamen; Cro, corolla; Crp, carpel; R, receptacle; and Clx, calyx.

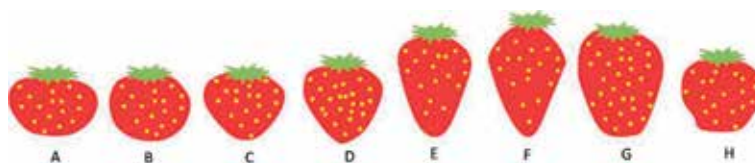
After fruit set, fertilizer should be applied as per schedule and special care should be given for disease prevalence. *Verticillium wilt* is a soil-borne disease for strawberry plants and precultivation fumigation with methyl bromide has been suggested to be the best remedy. However, methyl bromide has been banned due to its destructive effects in the ozone layer [11]. Foliar applications of micronutrients (trace elements) boost the plant growth, improve berry quality, and enhance crop yield. Boron application improves flower size, increases pollen quantity, and enhances root elongation. Magnesium deficiency is characterized by leaf marginal scorch and reduction in calcium causes immature and under-size fruit. These deficiency symptoms can be recovered by preflowering foliar application of magnesium nitrate and calcium nitrate. Depending on varietal characteristics and genetic makeup, the fruit with different morphological features is produced (Figure 3).

### 1.5 Biochemical changes during fruit ripening

As fruit matures, sucrose is continually provided by photosynthetic reactions. As a result, sucrose invertase activity increases during developmental stages. Accumulated sucrose in immature fruit tissues is hydrolyzed into monosaccharide (such as fructose and glucose). These three carbohydrates are major constituent of soluble sugars of fully ripened strawberries. It is estimated that nearly 150% of these accumulates have been observed as fruit fully ripens [12]. These massive transformations of primary metabolites initiate carbon fluxes into intricate and branchy biosynthetic pathways of secondary metabolites. This metabolic activity of fruit ripening can be observed by late accumulation of red pigmentation (pelargonidin 3-glucoside).

The fluctuation in the profile of bioactive compounds also depends on environmental cues. Temperature is positively correlated with soluble solid contents (SSC) and vitamin C; however, harvesting of late-season fruits showed inverse relationship between SSC and temperature. Similarly, ascorbic acid decreased gradually during postripening era. However, further studies have suggested that fluctuations in SSC content are independent from photosynthetically active radiation (PAR) [13, 14]. This phenomenon advocates that these events are driven genetically. Microarray analysis has demonstrated that gene expression plays key role in ripening of red (60% upregulation) and green (40% downregulation) fruits. *Polygalacturonase 1 (FaPG1)* is a fruit-softening gene that induces softening by disassembling cell wall, middle lamella, and consequently reducing the firmness [15].

Besides this, other dramatic changes in the content of fatty acids, organic acids, ketones, aldehydes, esters, alkanes, and sugars took place as fruit ripens. Biosynthesis of diverse volatile compounds and their precursors begins after full maturation of the fruit. As fruit color begins to change during ripening (green-to-red), emission of volatile compounds also initiated. Major volatiles reported in several studies include methyl ester, hexanoic acid, ethyl ester, butanoic acid, 1,6-octadien-3-ol,



**Figure 3.** Depending on morphological characteristics of strawberry fruits, it can be divided into eight shapes. A, oblate; B, globose; C, globose conic; D, conic; E, long conic; F, necked; G, long wedge; and H, short wedge.

4-methoxy-2,5-dimethyl-, 3,7-dimethyl-(-linalool), and 1,6-octadien-3-ol. It is estimated that more than 350 volatile compounds are produced for generating aroma and other organoleptic attributes in strawberry fruit [16–18].

## 1.6 Harvesting and handling management

The delicate heart-shaped strawberry is a nonclimacteric fruit and it ripens while it is attached to parent plant. Harvesting and handling operations are the key determinant of fruit quality. Loss of quality is due to high rate of metabolic activities and fruit sensitivity to fungal decay mainly gray mold (*Botrytis cinerea*) and/or rhizopus rot (*Rhizopus stolonifer*). Fruit is also very fragile to water loss, mechanical injuries, and skin bruising due to soft-textured berry without any protective rind (exocarp).

Strawberry should be harvested when fruit reaches appropriate shape and size according to varietal characters. Other biochemical parameters such as total soluble solid, vitamin C, and titratable acidity can also be assessed by taking field samples. The fruit can be harvested when skin colors fluctuate with 50–75% pink or red pigmentation, depending on local or distant markets.

After drying of dew drops, morning is the best time for harvesting strawberry fruit; and harvesting in afternoon should be avoided. Strawberry fruit intended for export consignments should not be harvested when temperature of berry exceeds 25°C. Since warm fruit might sustain significant pressure during handling, it needs more energy for precooling to remove the field-heat. After immediate rainfall, wet fruit should not harvest as it enhances the greater incidence of fungal diseases (*Botrytis gray mold*). Moisture and temperature are the major determinant of deciding harvesting time. Hence, many innovative growers cultivate strawberry crop under protected structures (tunnels or green houses) to hamper the fruit quality issues. Soft-textured fruits should be harvested on daily basis to avoid the deterioration.

The strawberry fruit should be picked with sharp knife while calyx (green leaves) is attached. However, for processing industry fruit can be harvested without calyx. The fruit can be picked by holding the stem (0.5–1.0 cm above the calyx) between index finger and thumb of right hand, followed by slight pinching of the stem. The fingers should not touch the fruit berry (fleshy part). The farmer or harvester should not wear any metal ring or any ornament to avoid the bruising. The slight squeezing of fruit cause release of sweet solution (sucrose) that acts as substrate for botrytis; hence damaged fruit should be separated immediately.

Generally, fruit packaging is done in the field in transparent box (250 or 500 g) right after harvesting. Picker should be trained enough to assess the fruit maturity stage, size, color immediately. Fruits with uniform size and color should be packed in same box. Diseased, unripen, mechanically injured, overripened, and skin-bruised fruit should be separated for different grades. Small packaging of strawberry fruits can be placed into big plastic crates. The crates can be loaded on picking carts. The picking carts hold the strawberry container in a horizontal position without rolling the fruit packaging. The packages are transported to field collection area or pack house.

## 2. Fruit harvesting and postharvest handling practices affecting strawberry fruit quality

### 2.1 Harvesting methods

Being a nonclimacteric fruit, strawberry fruits attain its highest quality features like normal size, regular shape, full red color, sweet taste, glossiness, firmness, and

aroma when fruits fully ripe while still attached to plant. This stage, for cultivar Chandler, one of the widely grown cultivar in subtropical climate, arrives between 28 and 35 days after the fruit set [19]. The onset of ripening stage is characterized by fruit changing color to red. A marketable strawberry fruit has healthy green calyx and stem. However, at this stage, the pericarp of the fruit is thin and delicate that needs a very careful and skillful harvesting method and, therefore, the fruits are harvested in the early morning when fruit temperature is low, and fruit is relatively firm [20].

For distant or export markets, relatively firmer fruits with  $\geq 75\%$  red color development are preferably picked to withstand rigors of transport. Although such fruits do not improve in taste, still they soften up and develop full red color. Strawberries are harvested in cooler part of the day when fruits are not wet and pulp temperature does not exceed  $25^{\circ}\text{C}$ ; otherwise, chances of bruising injury, shriveling, decay incidence, and high precooling cost will increase. Strawberry fruit along with its calyx is cautiously harvested by pinching the stem (0.5–1.0 cm above calyx) manually without squeezing the fleshy part of fruit. Grading and packing are normally carried out in the field. Overripe and too large fruits are not desirable as these fruits fetch low market value due to low cosmetic value.

Although, hand harvesting is the most widely used method of harvesting in strawberry, there have been several studies on mechanical and intelligent robotic harvesting methods. One of the first such systems was developed to harvest strawberries grown under hydroponics system [21]. Similarly, a computer vision-assisted robot system has been developed by [22] to detect and approach ripened strawberries grown under bench-type cultivation system. The programming was based on using real-time position tracking algorithms for fruit detection under natural light conditions. Further functions in this robotic system include gripping and cutting the fruit stem without damaging the strawberry fruit itself. Another such system for harvesting bench-type strawberries is based on machine vision, navigation system, and sonar technology to distinguish fruits according to hue and saturation histogram and the cutting area of stem was selected on the basis of binocular-vision system [23]. Improvement in fruit detachment and fruit classification method has been studied for robotic systems [24].

## **2.2 Losses due to physical and mechanical injury**

During and after harvest, soft fruits like strawberry are naturally prone to physical injury because these fruits have fragile epidermis. Mechanical damages are accompanied by morphological and physiological changes leading to adverse fruit sensory quality that may have economic repercussions. After harvest at ripening stage, strawberry fruits rapidly soften up owing to degradation of middle lamella due to pectin solubilization and hydrolysis of hemicellulose and cellulose [25]. During postharvest handling, strawberry fruits may get compressed, collide, punctured, or bruised leading to shorter shelf life and decay. This happens due to careless harvesting by untrained persons, piling harvested fruits, packing forcefully in rough container, handling fruit directly again and again, transporting in improper vehicle or on uneven road.

Bruising is the most undesirable damage caused that seriously limits not only the cosmetic value of fruits but also provides gateway to pathogens especially fungus [26]. Comparison between a sudden impact and slow compression on strawberries has shown that the fruit tissues are easily bruised by slow compression as sudden impact is absorbed by the fruit [27].

Therefore, proper standard procedures are followed in strawberry harvest to achieve economically profitable quality; otherwise, market value of the fruits drops sharply. To reduce such physical damage, strawberries are harvested by

trained persons and handled only once during the whole supply chain that is during harvesting, packed immediately after harvest in special containers and transported carefully in designed vehicle to reduce compression and jerks.

### **2.3 Temperature management**

Strawberry fruit is very sensitive to high temperature and at room temperature may survive up to 24–48 h with marketable quality [28]. Improper or no field heat removal and poor temperature management during the supply chain incur rapid quality deterioration and thus irreversible losses. After harvest, low temperature is the most appropriate strategy to maintain fruit quality [29] as rate of respiration increases exponentially from 28 ml CO<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> at 5°C to 127 ml CO<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> at 20°C [30]. As a first step, harvested fruits should be precooled immediately with forced air cooling or placing in refrigerated room as a delay of every 1 h between picking and precooling reduces 1 day of shelf or storage life at ~0°C [31, 32]. Therefore, to maintain strawberry fruit quality after harvest, the most common method is instant cooling of the fruits after harvesting and then storing continuously at low temperature range from 0 to 4°C [33]. Also, storing the fruit at temperature range between 0 and 1°C (32 and 34°F) and relative humidity from 90 to 95% increases its shelf life, minimizes physiological deterioration, and suppresses pathogenic decay incidence [32, 34].

Enzymatic and nonenzymatic antioxidant systems in strawberry fruit respond to low or high temperature in different ways. At higher temperature ( $\geq 5^{\circ}\text{C}$ ), the enzymes in strawberries that are involved in antioxidant mechanism viz. glutathione reductase, glutathione peroxidase, ascorbate peroxidase, guaiacol peroxidase, catalase, superoxide dismutase, monodehydroascorbate reductase, and dehydroascorbate reductase show greater activities compared to the enzymes in fruits stored at low temperature (0°C) [35]. Other important enzymes like RuBisCO are also significantly abundant in fruits stored in low temperature [36]. Antioxidant compound also behaves similarly, whereby glutathione, ascorbic acid, total anthocyanins, and total phenolic contents are higher in fruits stored either at higher temperature or for longer period. This also leads to higher antioxidant and radical scavenging activities in such fruits [35]. Low-temperature storage also effects higher fruit firmness, titratable acidity, total soluble solids, ascorbic acid content, and total terpenes in strawberry fruits; moreover, dehydration stress is more severe in strawberry fruits stored under room temperature [36].

### **2.4 Modified atmosphere packaging**

Strawberry fruits are seal-packed while modifying the air inside sealed packaging such that the air has high CO<sub>2</sub> concentration is called modified atmosphere packaging (MAP). This is relatively inexpensive technique used for maintaining fruit quality than CA storage. Generally, perforated (types include micro- and macroperforated) and nonperforated polymeric films are used in MAP as these films have specific and selective permeability for CO<sub>2</sub> and O<sub>2</sub>. Micro- and macroperforated packaging has shown promising results in sustaining the keeping quality and extending the storability of strawberry fruits [37].

Generally, recommended composition of MAP is 5–10% O<sub>2</sub>, 15–20% CO<sub>2</sub>, and 70–80% N<sub>2</sub> for strawberries [38]. Standard packing of fresh strawberry fruits is recommended by modifying internal atmosphere to 10% O<sub>2</sub> and 15–20% CO<sub>2</sub> and storage at 0–5°C, whereas for sliced strawberry fruit, few reports suggested to keep the concentration of O<sub>2</sub> at 1–2% and CO<sub>2</sub> at 5–10% and storage at 0–5°C [39]. Maintaining at least moderately low temperature is prerequisite for MAP storage as



respiration rate is greatly influenced by temperature than by gaseous concentration, with a 72–82% reduction in respiration rate if temperature was decreased from 23 to 10°C for various O<sub>2</sub> and CO<sub>2</sub> mixtures [40].

A computational model was developed for predicting the concentrations of O<sub>2</sub>, CO<sub>2</sub>, N<sub>2</sub>, and H<sub>2</sub>O in perforation-mediated polymeric packages during cold storage of strawberries [41]. The model incorporates respiration, transpiration, and diffusive transport of O<sub>2</sub>, CO<sub>2</sub>, N<sub>2</sub>, and H<sub>2</sub>O in microperforated package. The mathematical model depicted that the recommended concentrations of gases were marginally achieved at 5°C with 30- $\mu$ m-thick polymeric packaging with six microperforations each of 50  $\mu$ m diameter.

Polypropylene packaging with different perforation sizes has been used to naturally create mixture of gases in a concentration that resembles the concentrations used for modified atmospheric storage [42]. Polypropylene packages with different perforations stored strawberries at 2°C [43]. After 3 days, inside the packages, CO<sub>2</sub> concentration was between 1.5 and 2.6%, whereas O<sub>2</sub> levels were between 17.8 and 18.9% and this atmosphere composition remains stable for 1 week which caused maintenance of acceptable fruit quality for the first 10 storage days. Therefore, these perforated packaging was recommended due to less loss of marketable fruits, no signs of *Botrytis*-related decay and just a slight reduction in sucrose content.

However, modified atmosphere packaging alone is not fully capable to stopover infection of fresh strawberries by fungi such as *Botrytis mycosis* and *Rhizopus*. Therefore, prior treatment of strawberry fruits with different gases and coating before packing them in modified atmosphere packaging has been evaluated and found to be increasing the overall impact of MAP. MAP (2.5% O<sub>2</sub> and 15% CO<sub>2</sub>) combined with ozone pretreatment and an edible film coating extended shelf life up to 8–10 days compared to only MAP that increased shelf life by 4–6 days. All MAP treatments increased soluble sugar, ascorbic acid, acidity, and anthocyanin and decreased browning index and cell membrane permeability. Similarly, strawberries are pretreated with 30% CO<sub>2</sub> concentration for 3 h at 3°C before packaging the fruits in modified atmosphere packaging film, storing for 1 day at 1°C, transporting for 10 days at 1°C, and finally, distributing for 3 days at 4°C [44]. Treatment with CO<sub>2</sub> when combined with MAP maintained strawberry fruit quality by reducing weight loss, tissue softening index, and decay rate.

## 2.5 Controlled atmosphere storage

Generally, controlled atmosphere (CA) storage is characterized by increasing the concentration of CO<sub>2</sub> and decreasing that of O<sub>2</sub> in the ambient atmosphere of storage chamber [45]. However, change in composition of atmosphere is always accompanied by low temperature and high humidity during long-term storage. CA storage slows down rate of respiration, microbial infestation, and fruit-softening process, whereas suboptimum gaseous levels during CA storage may cause production of off-flavors and skin discoloration [25]. Most of the studies have suggested that CO<sub>2</sub> concentration between 15 and 20%, and O<sub>2</sub> concentration between 5 and 10% constitute the most suitable atmosphere composition for successful strawberry storage [46]. Lower or higher concentrations of oxygen such as 17,  $\geq$ 2, and 1.5% have also been recommended.

Strawberry fruits stored in CA retain better quality traits even at higher temperature (10°C) than fruits stored in air storage [47]. However, fruits stored at lower temperature (4°C) in CA storage show better quality than those stored at high temperature. Other than lowering respiration rate, one of the most important features of increased CO<sub>2</sub> level in CA storage is the excellent control of decay caused by *Botrytis* and *Penicillium* species [48]. Generally, strawberry fruit may be stored

for 10–14 day at 1°C with CA composition of 3–5% O<sub>2</sub> and 15–20% CO<sub>2</sub>. Too much concentration of CO<sub>2</sub> or too low concentration of O<sub>2</sub> produces off-flavors in strawberries. Other fruit quality features are also affected by CA storage. Strawberry fruits stored in CA storage retain firmness longer than just cold-stored fruits and rotting-related loss of fruits is also less in CO<sub>2</sub>-enriched storage. Strawberries stored in controlled atmosphere comprised of 2% O<sub>2</sub> and 12% CO<sub>2</sub> led to higher fruit firmness, titratable acidity, total soluble solids, and ascorbic acid content in strawberry fruits than fruits stored in air [36]. This study also found that controlled atmosphere storage maintained higher volatile concentrations than simple air storage. The esters and furanones, two of the most common volatiles produced during storage, were higher in CA-stored strawberries. Similarly, total terpenes, total acids, total alcohols, and tolerance to cold stress were also higher in strawberries stored in CA storage compared to air-stored strawberries. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a multifunctional enzyme that is stored during cold stress. CA storage led to significantly higher abundance of GAPDH and the expression of chloroplastic fructose-bisphosphate aldolase 3 (a protein of the Calvin cycle) was significantly higher in CA storage. Similarly, malic enzyme that is involved in carbon metabolism pathways had significantly greater abundance indicating potential capacity of CA-stored fruits for carbon fixation during senescence or photosynthesis.

## 2.6 Edible coatings

Edible films or coatings are nontoxic, environment friendly, and food grade formulations used to maintain postharvest quality and increase shelf- and storage life of fruits and vegetables. These formulations are also used as active packaging to reduce dehydration, microbial attack, skin browning, and tissue softening. Edible coatings are applied in solution form, whereas edible films are first molded into sheets and then wrapped around food products. Edible films or coatings are based on carbohydrates, protein, lipids, or their different combinations. For example, chitosan, pullulan, alginates, starches, and pectin have been widely studied edible coatings or films [49, 50]. Recent researchers have shown that beneficial effects and easy handling of edible coatings and films make their use a preferable choice for preserving fruits and vegetables, especially for soft fruits such as strawberries. The bioactive and functional benefits of edible coatings include slow rate of respiration and tissue softening, extended postharvest life, biodegradability, and lower microbial infestation [50–52].

Pullulan and alginate are other polysaccharides that have been extensively used as edible coating [53, 54]. Chitosan, alginate, and pullulan were applied on strawberry fruits stored at 4°C to evaluate the effect of these coatings on fruit quality and its antioxidant system. They found that these polysaccharides-based coatings delayed tissue softening and decay, loss of total soluble solid, titratable acidity, ascorbic acid, and total phenolic contents. Additionally, polysaccharide coatings also enhanced the activities of superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase that prevented lipid peroxidation and reduced membrane damage. Combinations of coatings such as carboxymethyl cellulose and hydroxypropylmethyl cellulose exhibit better retention of fruit quality [55, 56]. *Aloe vera* gel and gum Arabic also play significant role for improvement of shelf life [33, 57].

The essential oils are bioactive compounds used as additives to edible coatings for enhancing the ability of edible coatings to preserve fruit quality and reduce microbial spoilage. Essential oils of oregano, red thyme, peppermint, and lemon-grass incorporated in chitosan coatings on strawberry fruits have shown antifungal properties and reduced the number of decayed fruits during storage, thus extending storage life of fruits at 4°C [58]. Similarly, strawberry fruits coated with alginate and pectin-based coatings loaded with citral and eugenol showed higher values for

firmness, total soluble solids, and antioxidant activity, and lower value for weight loss and microbial spoilage [59]. Strawberry fruits coated with the combination of oleic acid [60], sodium benzoate and potassium sorbate [53], and calcium salts [61] with chitosan have shown improvement in postharvest quality and life.

Studies conducted to compare the effectiveness of chitosan (practical grade) in combination with different acids (like formic, glutamic acetic, and hydrochloric acid), and commercially applied water dissolving chitosan formulation to control various types of postharvest diseases of strawberry fruit such as blue mold, gray mold, and Rhizopus rot mold during 4 days' storage at  $20 \pm 1^\circ\text{C}$ . However, commercial grade chitosan showed good control of strawberry diseases kept 7 days ( $0 \pm 1^\circ\text{C}$ ) and 3 days' shelf life. Furthermore, it was noticed that the treatment ( $0 \pm 1^\circ\text{C}$ ) which is experimentally resistant induces effectively in controlling blue mold, gray mold, and Rhizopus rot stored for the 7 days of storage of strawberry fruit which thereafter exposed to 3 days shelf-life [62, 63].

The effect of a novel edible biofilm containing *Cryptococcus laurentii* ( $109 \text{ cfu ml}^{-1}$ ) in combination with glycerol, palmitic acid, alginate, glycerol, glycerol monostearate, and  $\beta$ -cyclodextrin to reduce the incidence of diseases and to extend the postharvest life of strawberries by applying sodium alginate film containing the *C. laurentii*, effectively controlled microbial decay, maintained the quality and firmness, reduced weight loss thus enhancing the shelf life and storage properties of strawberry fruit [51]. The candelilla wax alone and in combination with a (*Bacillus subtilis*) HFC103 strain on postharvest life of strawberry fruit kept for 6 days at  $25^\circ\text{C}$  by [64]. Reduction in weight loss and decay percentage in comparison to control since 3 days was observed in the treatments having bacteria and film alone and combination of both. However, reduced decay percentage was recorded around 100% in film + bacteria treatment, as compared with control on day 6 significantly reduced the severity index in film followed by bacteria and film + bacteria treatments.

## 2.7 Chemicals treatments

Strawberry is perishable fruit which is highly susceptible to different postharvest losses (50%) due to sudden attack of fungal diseases. For a decade, various kinds of synthetic chemicals have been utilized to increase postharvest life; however, their uses are highly restricted due to food safety issues. Alternative strategies such as heat treatments can be used to enhance the postharvest storage life by increasing natural resistance and antioxidative systems in postharvest technology. 1-Methylcyclopropene (1-MCP) was applied on strawberry cv. Everest tended to maintain firmness and color, and higher level of disease was observed in fruit treated at higher application rates of 1-MCP. 1-MCP showed reduction in advancement of anthocyanin and phenolic contents and inhibited phenylalanine ammonia-lyase (PAL) activity. Higher level of 1-MCP application to strawberry fruits might lower disease resistance in strawberry fruits [65].

Two varieties of strawberry fruit including Camarosa and Red Dream and two raspberry cultivars Nova and Killarney fruits were harvested and dipped in (2.5 min) into ascorbic acid solution (0, 1 or 2%), frozen at  $-40^\circ\text{C}$  using plastic containers for storage at  $-20^\circ\text{C}$  for 6 months. Total soluble solids, pH, and total phenolic contents decreased less in treated fruits than control in the same rate in strawberry and raspberry cultivars after 3 months of storage periods. Polyphenol contents increased in ascorbic acid treatments, while increasing trend in different in cultivars [66].

Frozen strawberries show changes in color and texture degradation which affects the overall quality attributes. Fresh strawberry was dipped alone or in combination of calcium lactate and citric acid during freezing process. Citric acid clearly lowers browning index and improves the ascorbic acid and total anthocyanins content

(TAC), while calcium lactate in texture maintenance and firmness after thawing. The combination of both citric acid and Ca lactate (0.4% + 1%) showed better quality characteristics, like maintaining vitamin C content, firmness, anthocyanin content while reduction in drip loss improving in color attributes in comparison with other treatments [67].

Application of melatonin to strawberry fruits significantly reduced decay, weight loss, and senescence of strawberry fruit during storage [68]. It was further observed that melatonin also affected the various fruit quality attributes like fruit color, firmness of fruit, total soluble solids content (SSC), and titratable acidity (TA) of the fruit. Treated fruits showed higher antioxidant capacity, and reduced the hydrogen peroxide and malondialdehyde. Moreover, melatonin improves the quality attributes, extends postharvest life and expression of melatonin biosynthetic genes, and increased amount of internal melatonin.

## 2.8 Heat treatments

In the previous studies, the effect of heat treatments has also been checked for improvement of quality attributes and postharvest life of strawberry fruits. Heat-treated strawberry fruit treated with heat at 45°C for 3 h showed high firmness, reduced activities of enzyme like  $\beta$ -Xyl 9  $\beta$ -xylosidase), EGase (endo-1,4- $\beta$ -d-glucanase) which delayed hemicellulose deprivation in both zones (external and internal fruit zones). In addition, inhibited polygalacturonase (PG),  $\beta$ -galactosidase ( $\beta$ -gal) activity, and elevated the PME activity with higher EDTA soluble pectins in heat-treated fruits than the control. Moreover, these activities varied in exterior and inner surface of fruits which could affect solubilization of hemicelluloses and pectins [69].

Reduced decay in heat-treated strawberries (45°C, 3 h) was noticed during storage period in comparison to control [70]. It was also seen that heat-treated fruits also have lower levels of H<sub>2</sub>O<sub>2</sub> than control fruit during storage. In addition, higher antioxidant capacity and enzymes activities (APX, SOD) were recorded in heat-treated fruit during the course of storage which representing changes in the oxidative metabolism of the fruit. Moreover, heat-treated fruits showed differential responses during storage which could save the fruit against reactive oxygen species (ROS) produced during senescence or invasion of pathogen. Three different strawberry cultivars including Dover, Campineiro, and Oso Grande harvested and kept for 6 days at 6, 16, and 25°C by [31]. Strawberry varieties presented variation in chemical composition at different storage temperatures and these differences might be concerning regarding their adaptation to low temperatures.

However, some quality attributes are negatively (anthocyanin and vitamin C) affected to low temperature, some are positively (soluble sugars) affected, while some bioactive compounds remain same or decreased like ellagic acid, flavonols, and total phenolic contents (TPC) during storage conditions. Few reports suggested that heat-treated strawberry fruits (*Fragaria ananassa* Duch., cv Camarosa) showed higher firmness of fruit and lower appearance of expansin genes (FaEXP1, FaEXP2, and FaEXP6) during the following 24 h after which might contribute to delay softening in strawberries [71].

## 2.9 Essential oils

Essential oils have strong antimicrobial activities and have been incorporated in edible coatings and films not only to improve texture of coatings but also as antimicrobial agents. Essential oils of clove, cinnamon, and oregano were used in

paraffin coatings of paper packaging materials that totally inhibited the growth of *Candida albicans*, *Aspergillus flavus*, and *Eurotium repens* on strawberry fruits for 7 days at 4°C [72].

In the recent years, numerous researches have been initiated for new alternative technologies to preserve foods which are prime interest to the fast-growing food industry. Essential oils of clove and/or mustard in vapor phase were evaluated in vitro and in vivo on strawberries against *Botrytis cinerea*. Essential oils showed good results in combination rather than individual application. Essential oils exhibited inhibitory activity which is due to major compounds presented in the mustard and clove oils and reduction in the development of *Botrytis cinerea*. It has synergistic antifungal effect which is more effective in combination compared to individual essential oil application [73]. Studies have shown that there is an ecofriendly and consumer-acceptable approach for pathogen control on various fruits like strawberries during postharvest storage. Application of *cassia* oil in 400–800 ppm concentration effectively repressed the growth of *E. coli* in vitro and *Botrytis cinerea* on treated strawberries [74]. It also strongly inhibited the enlargement of germ tube and spore germination at an application rate of 100 ppm. Cassia oil application reduced decay percentage, weight loss in strawberries during postharvest storage.

Tea tree oil (TTO) was applied before harvest on strawberries to investigate the physicochemical properties and quality parameters. TTO reduced the decay, and delay firmness, decreases number of microorganisms during storage. It also reduces the accumulation of H<sub>2</sub>O<sub>2</sub> with increasing antioxidative activities of various enzymes including catalase and ascorbate peroxidase as well as β-1,3-glucanase. TTO-treated fruit showed 188 differentially expressed proteins as compared with control. Of these, 29 were abundant, 159 are less abundant, and 3 proteins related to cell metabolism were downregulated, and 4 proteins related to stress were upregulated in fruit treated with tea tree oil. TTO application before harvest significantly reduced decay, microorganisms, delays fruit senescence, and improves the defense proteins [75]. Chitosan (CH) and carboxymethyl cellulose (CMC) coatings enriched with *Mentha spicata* essential oil (MSO) can be utilized in food industry as suitable active packaging materials for the preservation of fresh strawberries. The effects of edible coatings of CMC and CH in combination with essential oils of MSO (0.1 and 0.2%) on microbial growth, sensory and physicochemical attributes of fresh strawberries fruit during refrigerated storage conditions [56]. It has been found that 0.2% CMC-MSO proved best in organoleptic and physicochemical attributes as well as microbial inhibition during storage period [56].

## 2.10 UV irradiation

UV-C irradiation plays an important role in reducing fruit decay and delaying ripening of fruits. It has been used extensively before storage at relatively higher doses due to their higher impact and use. Strawberry fruits were subjected to different repeated doses of UV-C irradiation for quality maintenance during storage. UV-C irradiation reduced decay, weight loss, and softening in strawberries. Two-step or multistep UV treatments showed higher quality retention and reduced calyx browning more efficiently. UV-C treatments could be more effective to enhance the postharvest life of strawberry during storage conditions [76]. Also, studies showed that UV-C radiation increased in total phenolic, volatile contents, proanthocyanidins, anthocyanins, and esters in external tissues. However, aroma character compounds decreased with the application of UV-C treatments [77].

Strawberry fruits were subjected to blue light to improve quality, antioxidant capacity, and enzyme activities stored at 5°C. Blue light illumination

increased ascorbic acid, total sugar, titratable acidity, total phenolic, and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activity in strawberries during the course of storage. In addition, higher activities of reactive oxygen species like APX, SOD, and CAT maintained lower amounts of superoxide anion ( $\text{SO}_2$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), and malondialdehyde (MDA). Thus, for maintenance of quality attributes and improvement in nutritional quality of strawberry fruit, exposure to blue light illumination might be affective due to the enhancement of their antioxidant systems and free radical-scavenging abilities [78]. Strawberry fruits were exposed to the lower dose gamma irradiation at 1 kGy and different amounts of EMAP (active equilibrium-modified atmosphere packaging) at the rate of EMAP1:  $\text{CO}_2$  10%:  $\text{O}_2$  5%:  $\text{N}_2$  85% and EMAP2:  $\text{CO}_2$  5%:  $\text{O}_2$  10%:  $\text{N}_2$  85%, and stored at 4°C. EMAP1 packages showed good texture, appearance, and firmness than EMAP2 during storage time. It has been noted that the exposure to lower irradiation dose in combination with EMAP1 maintained external appearance, less fungus attack, and enables high-quality strawberry with improved shelf life [79].

### **3. Conclusions**

Strawberry is a highly perishable fruit and subjected to several postharvest losses after harvest. There are several stages which are responsible for quick losses such as improper harvesting methods, developmental stage, improper picking time, sorting and packaging, transportation postharvest treatments, and storage conditions and as well as untrained labor. However, numerous pre- and postharvest studies were conducted to develop strategies for extension of strawberry shelf life such as harvesting methods, heat treatments, UV-C irradiations, coating, and essential oil applications. Furthermore, there is need a to study these technologies on commercial scale to increase the net income. There is a need to develop ecofriendly alternative technologies to enhance the shelf life of strawberries.

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# Improving Yield and Antioxidant Properties of Strawberries by Utilizing Microbes and Natural Products

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## Abstract

Consumption of strawberry has gone up worldwide due to its proven health benefits. Strawberry growers are using synthetic fertilizers and pest management products to maximize yield. This situation posed a risk by affecting sustainability of strawberry production and tainting reputation of a healthy fruit by placing it in the list of dirty dozen due to pesticide residues on fruit. Alternative approaches for increasing yield and pest management of strawberry to minimize environmental and health hazards are possible. Recent studies on alternative natural products (e.g., chitosan) and beneficial microbes (e.g., *Bacillus*, *Paraburkholderia*, etc.) indicated that growth, yield, and fruit quality enhancement are supported by these products and may help in sustainable strawberry production. This chapter reviews and updates our knowledge on the health benefit of strawberry and research findings on the use of natural products and probiotic bacteria for yield and quality improvement in strawberry.

**Keywords:** probiotic, antioxidants, sustainability, disease control, strawberry yield, microbial biostimulant

## 1. Introduction

Strawberries are a popular fruit in the US and worldwide. In the US, per capita consumption of strawberries has increased from 2 pounds/person/year to approximately 8 pounds/person/year in recent years [1]. This trend is also apparent in other developed and developing countries of the world. As a result, strawberry growers are using synthetic fertilizers and pest management products to maximize yield. As increased use of synthetic chemicals (fertilizers and pesticides) in crop production and protection has posed a threat to both environment and human health [2], an alternative approach for plant growth promotion, pest management, and sustainable agriculture is being explored all over the world. Strawberry and other fruits and vegetables that are mostly consumed fresh are getting special research attention to innovate production techniques excluding synthetic chemicals [3]. Strawberry growers are specifically eager to find new agro-techniques with special emphasis on the use of both plant growth promotion and nutritional quality improvement in a move toward a more sustainable and environment-friendly approach. In addition,

researchers have been testing novel, sustainable approaches to improve the quality and antioxidant properties of strawberries to increase health benefits. One of the reasons for strawberry demand and consumption has been going up as this fruit is an excellent source of natural antioxidants, such as carotenoids, phenolics, vitamins, anthocyanins, and flavonoids with remarkably high capacity of scavenging free radicals [4]. Improving fruit quality and yield sustainability without synthetic inputs is a research priority for this nutritious fruit. Beneficial microorganisms that are used as bio-fertilizers or bio-stimulants possess the ability to colonize the rhizosphere, plant roots, or both when applied to seeds or plant organs that are used for vegetative propagation (strawberry tips). Some of these microbes have shown potential to promote strawberry plant growth by the release of metabolites into the rhizosphere that may inhibit various pathogens as biocontrol agents [5–8]. However, Tomic et al. [9] found that the response to bacterial inoculation is cultivar-related in strawberries. These microbes were reported to improve plant nutrition and support plant development under natural or stressed conditions as well as increase yield and quality of many important crops and thus may play a crucial role in sustainable crop production in the future [10–12]. A small but significant body of literature also suggests that these microbes can increase strawberry fruit quality in terms of taste and nutritional value and thereby have a positive impact on human health with associated reduction of healthcare costs [13, 14]. The objective of this review is to update our knowledge on the research conducted on improving yield and quality of strawberry by using natural products and beneficial microbes around the globe. Major focus of the review is to relate bio-fortified strawberry fruit with human health benefit. Some novel eco-friendly approaches and potential mechanisms involved with yield and quality improvement in strawberry are also discussed.

## **2. Nutritional and health benefit profile of strawberry**

Strawberries are an excellent source of essential and health benefitting nutrients (**Table 1**) and low in total calories with a 100 g serving providing only 32 kcal. Their sweet flavor makes them a delicious alternative to processed foods. Dietary fiber present in strawberries may contribute to regulating blood sugar levels by slowing digestion. Fiber content may also control calorie intake by its satiating effect. Strawberries contain fat-soluble vitamins (i.e., vitamin A and tocopherol) and carotenoids (i.e., lutein and zeaxanthin), but one of the aspects of major nutritional relevance is the extremely high content of vitamin C, even higher than citrus fruits. Together with vitamin C, folate plays a crucial role in the nutritional quality of strawberry as it is one of the richest natural sources of this essential micronutrient, and folate is an important factor in health promotion and disease prevention [15, 16]. Strawberry is a source of several other vitamins such as thiamin, vitamin B6, vitamin K, vitamin A, and vitamin E although to a lesser extent (**Table 1**). It is also an excellent source of manganese providing more than 20% of the daily adequate intake (AI) for this mineral per serving. The same amount of strawberries can provide about 5% of the AI for potassium and is known as a good source of iodine, magnesium, copper, iron, and phosphorus (**Table 1**).

### **2.1 Role of strawberry as a source of dietary antioxidants compared with similar sources**

Strawberry consumption can help to prevent inflammation, oxidative stress, cardiovascular disease (CVD), certain types of cancers, type 2 diabetes, and obesity. The addition of berries to the diet can positively influence risk factors for CVD by



Type	Nutrient	Per 100 g
Minerals	Calcium (mg)	16
	Iron (mg)	0.41
	Magnesium (mg)	13
	Phosphorus (mg)	24
	Manganese (mg)	0.386
Vitamins	Vitamin C (mg)	58.8
	Folate ( $\mu$ g)	24
	Thiamin (mg)	0.024
	Lutein + zeaxanthin ( $\mu$ g)	26
	Vitamin E, $\alpha$ -tocopherol (mg)	0.29
	Vitamin K ( $\mu$ g)	2.2
	Vitamin B6 (mg)	0.047
Proximates	Dietary fiber (g)	2.0
	Fructose (g)	2.44

*Adapted from [17].*

**Table 1.**  
*Nutrient composition of fresh strawberries.*

inhibiting inflammation, improving plasma lipid profiles, scavenging free radicals, and increasing LDL resistance to oxidation [18]. The mechanisms by which strawberries exert these positive effects are not completely understood. Among many potential mechanisms, its role as an antioxidant is the most relevant as strawberry supplementation significantly decreases oxidative stress, protecting mononuclear blood cells against DNA damage [19, 20]. Several studies have shown that strawberry generally possesses a high level of antioxidant activity, which is linked to the levels of phenolic compounds in the fruit rather than vitamin C [1, 21–23]. Wang and Jiao [24] showed that strawberry juice extracts exhibited a high level of antioxidant capacity against free radical species. Strawberry extracts also seem to modulate cell signaling in cancer cells by inhibiting proliferation of several types of cancer cells inducing cell cycle arrest and apoptosis and suppressing tumor angiogenesis [25]. An unavoidable result of aerobic metabolism in humans and other organisms is the production of reactive oxygen species (ROS). ROS include free radicals such as the superoxide anion ( $O_2^{\bullet-}$ ) and hydroxyl radical ( $\bullet OH$ ), as well as nonradical molecules like hydrogen peroxide ( $H_2O_2$ ), singlet oxygen ( $^1O_2$ ), etc. All ROS can be damaging to organisms at a concentration where its level exceeds the defense mechanism. These excess ROS can put cells in oxidative stress that eventually pose a threat to cells by causing peroxidation of lipids, oxidation of proteins, damage to nucleic acids, and enzyme inhibition. The enhanced production of ROS during physiological stresses can also activate a programmed cell death (PCD) pathway that may lead to cell death [26–33]. Under normal conditions, ROS molecules are unable to cause any damage as they are constantly being scavenged by a range of antioxidative mechanisms [34]. But, the delicate equilibrium between the ROS production and their scavenging by antioxidants is disturbed by multiple stress factors. An efficient antioxidative system that includes nonenzymatic as well as enzymatic antioxidants in a cell can usually scavenge or detoxify excess ROS [35]. The human antioxidant defense system includes endogenous (enzymatic and nonenzymatic) antioxidants and exogenous antioxidants such as vitamin C, vitamin E, anthocyanidins,

carotenoids, flavonols, and polyphenols, with the diet being the main source [36–39]. Exogenous antioxidants play a key role in this delicate equilibrium between oxidation and antioxidation in living systems [36, 37, 40, 41]. Under physiological conditions, the human antioxidative defense system allows the elimination of excess ROS. However, our endogenous antioxidant defense systems are incomplete without exogenous reducing compounds such as vitamin C, vitamin E, carotenoids, and polyphenols. Therefore, there is a continuous demand for exogenous antioxidants to prevent oxidative stress.

Strawberry polyphenolic phytochemicals perform nonessential functions in plants but have large impacts on humans. Of the polyphenolic compounds, anthocyanins in strawberries are the best-known and quantitatively the most important. Studies have determined total anthocyanin content as 150–600 mg/kg of fresh weight. [17]. Strawberries also contain small amounts of other phenolic compounds as shown in **Table 2**. Evidence from in vitro studies shows that strawberry phenolics may have anti-inflammatory effects and suppress mutagenesis through antioxidative and genoprotective properties. Additionally, the content and composition of flavonols have been studied [42], and these compounds are identified as derivatives of quercetin and kaempferol, with quercetin derivatives being the most abundant [43]. The contents of the flavonoid groups, flavonols, and anthocyanins in strawberry extracts have been associated indirectly and directly, respectively, with the total antioxidant capacity for low-density lipoproteins [21]. Flavonoids in strawberries exhibit antioxidant [44, 45] and anticancer properties as well [46]. Elevated levels of these secondary metabolites should provide better health benefits to the consumers of strawberry.

Among numerous studies conducted on antioxidant contents in fruits and vegetables, results have shown that strawberry possessed a high level of antioxidant activity compared with others in the same group, and the activity was directly linked to the levels of phenolic compounds in the fruit [1, 21, 22]. A comparative study on the antioxidant activity of strawberry extract with other fruits based on the oxygen radical absorbance capacity assay indicated that its antioxidant capacity was higher than extracts from plum, orange, red grape, kiwifruit, pink grapefruit, white grape, banana, apple, tomato, pear, and honeydew melon [47]. However, Sun et al. [22]

Class	Group	Compound
Flavonoids	Anthocyanins	Cyanidin-3-glucoside
		Cyanidin-3-rutinoside
		Pelargonidin-3-glucoside
		Pelargonidin-3-rutinoside
		Pelargonidin-3-malylglucoside
	Flavonols	Quercetin-3-glucuronide
		Quercetin-glucoside
		Kaempferol-3-glucoside
		(+)-catechin
Phenolic acids	Hydroxycinnamic acids	Proanthocyanidin B1 (EC-4,8-C)
		Proanthocyanidin B3 (C-4,8-C)
		p-coumaroyl hexose

*Adapted from [17].*

**Table 2.**  
*Polyphenol composition reported in strawberries.*

ranked fruit differently for antioxidant contents based on total antioxidant oxyradical scavenging assay. These results put strawberry behind cranberry, apple, and grape but before peach, lemon, banana, pear, orange, grapefruit, and pineapple in terms of antioxidant activity of fruit extracts. Total antioxidant activity of strawberry can also relate to the contents of anthocyanins, which are typically present at high levels in this fruit [47]. Great interest has developed in strawberries due to the extremely high content of vitamin C, which makes them an important source of this vitamin for human nutrition. Relatively high content of ellagic acid is also a reason of interest for strawberries to consumers. Ellagic acid is an antioxidant that has been proposed to exert antimutagenic and anticarcinogenic effects [48, 49]. Nutritional quality of strawberry is reflected in its high levels of vitamin C, folate, and phenolic constituents [17], most of which show relevant antioxidant capacities *in vitro* and *in vivo* [50]. Moreover, strawberries are economically feasible and commercially important, and are widely consumed as fresh or in processed forms such as jam, juice, and jelly. Due to the high nutritional quality, taste, and health benefits, strawberries are among the most studied berries from the aspects of horticultural, genomic, and sustainable production practices.

### **3. Enhancement of yield and antioxidant contents in strawberry by various natural products including chitosan**

To overcome the challenge of increasing strawberry production with a significant reduction of agrochemical use and environmental pollution (especially from synthetic chemicals), a great deal of interest and research has been devoted to natural products and beneficial microbes in recent days. Many growers and researchers are actively looking for ways to create a more sustainable production system through use of natural inputs while simultaneously improving yield and antioxidant properties. A large body of literature suggested that integration of these products with conventional management tools could significantly reduce chemical use and make strawberry production more sustainable. Various natural products have been tested in strawberry production to improve yield and quality by preventing disease and stimulating growth and development. Among the natural products, chitosan is the most tested that has shown growth and yield stimulating effect together with efficacy against diseases in strawberries and other crops [51]. Chitosan is a polysaccharide derived from chitin outer skeletons of shell fish and crustaceans such as crab, crayfish, lobster, and shrimp. As chitin is deacetylated by sodium hydroxide to obtain chitosan, it is slightly basic and is soluble in dilute aqueous acidic solution ( $\text{pH} < 6.5$ ). Once dissolved, it can be further diluted with water to apply on plants at all different growth stages. In general, it is nontoxic to humans and considered safe for agricultural uses due to its quick degradation in the environment. Once chitosan or its derivatives come in contact with plants, they bind with the cell plasma membrane and elicit defense responses through expression of pathogenesis-related (PR) genes, accumulation of phytoalexins, callose, oxidative burst, and formation of reactive oxygen species. Expression of these PR genes and accumulation of antimicrobial phytoalexins are believed to play a major role in controlling pre- and postharvest pathogenic diseases. A large body of published reports supports antimicrobial activities of chitosan against a wide range of phytopathogens [52]. Similar studies also found that the biostimulant chitosan promoted plant growth and development and provided enhanced disease suppression capability to plants through multiple mechanisms including induced systemic resistance [51, 53]. Chitosan has been widely used as fruit coatings to enhance storability and preserve anthocyanin and other antioxidants in strawberry [51], and various other fruit mainly for protection

from postharvest losses due to microbial infections [51, 54]. In addition, many investigators reported that chitosan use as a foliar spray increased vegetative growth, yield, and biochemical contents in plants [55–58]. Improvement of yield and functional properties of strawberry fruit through application of chitosan should be considered a sustainable option. A recent study by Rahman et al. [58] showed that multiple application of low concentrations (ppm level) of chitosan on the canopy of field grown strawberry plants at the prebloom stage significantly improved growth and yield. Authors also reported concurrent increase in various antioxidant contents and total antioxidant activities in treated fruit compared to nontreated control. This is an interesting and significant finding as total antioxidants and pigments such as anthocyanins are determinants of health benefits of strawberry fruit. Rahman et al. [58] also determined the effect of different doses of chitosan biopolymer on growth, fruit yield, and human health benefiting antioxidant properties of strawberry and found that both yield and contents of antioxidants are increased in a dose-dependent manner to some extent compared to untreated control. These findings indicate that the biostimulant chitosan can be an attractive agent for production of high quality and human health benefiting strawberry [58]. Results also indicated that foliar application of varying doses of chitosan on strawberry canopy stimulated all aspects of vegetative growth (plant height and root length) that may have influenced fruit yield and fruit quality compared with untreated control (Table 3). These findings were also interesting as all doses of chitosan improved growth of strawberry plants to some extent and may be experimented in similar crops being grown in soils with varying physical, chemical, and biological characteristics. This study was one of the few of its kind that determined the effects of natural products such as chitosan application on field-grown strawberry plants influencing yield and contents of multiple antioxidants in fruit. Experimental protocol for this study can be found in Rahman et al. [58].

Among a few different chitosan concentrations tested in the study, 500 ppm provided the highest fruit yield (42% higher than untreated control) in “Strawberry Festival” compared with untreated control (Table 3). Similar to yield response and a few other antioxidants, chitosan spray application on the canopy of strawberry also significantly increased fruit anthocyanin contents in a dose-dependent manner that plateaued at 500 ppm with 184.3 mg cyanidin-3-*O*-glucoside/100 g fruit. This increase of anthocyanin contents was equivalent to 2.3-fold higher compared

Treatment	Plant height (cm)	Root length (cm)	Total fruit weight/plant (g)	Total anthocyanin content	Total phenolic content	Total antioxidant activity
Control	19.5 ± 1.0b	19.25 ± 0.4c	246.6 ± 0.4d	81.11 ± 0.9d	310.4 ± 0.7c	250.9 ± 0.9c
Ch 125	20.41 ± 0.9b	21.16 ± 0.2bc	317.5 ± 0.7c	83.1 ± 1.0cd	356.5 ± 1.0b	252.6 ± 1.0c
Ch 250	21.75 ± 0.8b	22.66 ± 0.7ab	325.7 ± 0.5c	94.6 ± 0.5c	317.8 ± 0.5c	358.6 ± 1.0b
Ch 500	25.1 ± 1.0a	24.33 ± 0.2a	351.25 ± 0.5a	184.3 ± 1.9a	363.2 ± 0.4ab	374.42 ± 1.0b
Ch 1000	24.91 ± 1.5a	24.16 ± 0.6a	337.7 ± 0.4b	163.9 ± 0.6b	370.9 ± 0.4a	415.6 ± 0.5a

Five different concentrations, 0, 125, 250, 500, and 1000 ppm, of chitosan solution were prepared by dissolving the required amount in 0.1 N HCl and diluting with distilled water with pH adjusted at 6.5 by NaOH. Freshly prepared chitosan solutions were applied onto strawberry plants in each experimental unit prior to flowering and at 10% flowering stage by spraying up to run off at five different times with 10-d intervals. Cumulative fruit harvest from each plot was recorded. The required amounts of fruit tissues from first harvest were subjected to analyses for phenolics and other antioxidants mentioned in the table. Values are means ± standard errors of three independent replications ( $n = 3$ ). Different superscripted letters within the column indicate statistically significant differences among the treatments according to Fisher's protected LSD (least significance difference) test at  $p \leq 0.05$ , adapted from [58].

**Table 3.** Effect of chitosan application on yield and content of antioxidants in strawberry fruit.

with untreated control. The fruit produced by the plants treated with 1000 ppm chitosan solution in the study by Rahman et al. [58] had the highest total phenolic content (370.9 µg gallic acid/g fruit) indicating that chitosan concentration should be adjusted depending on the intended quality improvement in strawberry fruit. Total antioxidant activities of strawberry fruit obtained from both varied rates of chitosan treated and untreated control plants were assayed by utilizing the DPPH method, and the results were expressed as butylated hydroxytoluene (BHT) equivalents per gram of strawberry fruit. The highest total antioxidant activity was quantified in strawberry fruit obtained from 1000 ppm chitosan (415.6 µg BHT/g fruit) treated plants. These results reveal that application of chitosan on the canopy of strawberry could increase antioxidant activity in fruit up to 1.7-fold compared to untreated control (**Table 3**) [58].

A few other examples of natural products that have been investigated on strawberry with varying results are derived either from seaweed or compost. Seaweed products are used as nutrient supplements, biostimulants, and biofertilizers to augment plant growth and yield in agriculture. A study by Masny [59] found no effect on disease suppression of *Botrytis cinerea* on strawberry by applying seaweed products. However, application of these products had a significant influence on yield with an increase in the range of 17–42%. Compost or tea extracts were also used for plant disease control and for plant nutrition and growth promotion. Welke [60] assessed the effect of compost extract application on strawberry. Aerobically prepared extracts were effective in both disease suppression (*B. cinerea*) and increasing yield compared to the control.

#### **4. Strawberry growth, yield, and quality improvement by probiotic bacteria**

Beneficial microorganisms especially bacteria that are associated with host plants either as rhizoplane, phylloplane, or endophyte and enhance growth of the host plants including yield are popularly known as plant probiotic bacteria (PPB). These PPB can also suppress plant diseases by various modes of action when applied proactively in adequate amounts [61]. PPB that are used as biofertilizers or biostimulants possess the ability to colonize the rhizosphere, plant roots, or both when applied to seeds or crops. Some of these microbes have shown potential to promote strawberry plant growth by the release of metabolites into the rhizosphere that may inhibit various pathogens as biocontrol agents [5–8]. However, Tomic et al. [9] found that the response to bacterial inoculation is cultivar-related in strawberries, which indicates that a specific microbial strain should be tested for efficacy against a specific strawberry variety before large scale use. Microbes belonging to this group are also known as plant growth promoting rhizobacteria (PGPR) and were reported to improve availability of plant nutrient and support plant development under natural or stressed conditions as well as increase yield and quality. Although beneficial microbes have not been widely researched or used for improving yield and quality of strawberry, a large body of evidence indicates that many available beneficial microbes were found to provide growth and yield enhancement to diverse crop commodities [10–12], which can be tested for similar efficacy on strawberry and thus may play a crucial role in sustainable strawberry production in the future. A significant body of literature suggests that these microbes can increase strawberry fruit quality in terms of taste and nutritional value and thereby have a positive impact on human health with associated reduction of healthcare costs [13, 14]. A few relevant examples of positive effects of antagonistic microbes on multiple crops include protection against *Verticillium dahliae* [62] and protection of tomato

against *Alternaria solani* [63]. Some of these microbes were used in vitro and should be evaluated in vivo or in field conditions. For example, in vitro-beneficially-bacterized plantlets of grapevine not only grew faster than non-bacterized controls but also were sturdier, with a better developed root system and significantly greater capacity for withstanding gray mold fungus [64]. Similarly, banana plantlets treated with endophytes *Pseudomonas* and *Bacillus* species showed improved vegetative growth, physiological attributes, and strong defense against bunchy top diseases in the field [65, 66]. Seed treatment or augmenting beneficial microbial population in soil was also found to reduce seedling mortality from soil-borne diseases [67]. Biological agents such as *Trichoderma*, *Serratia*, and *Pseudomonas* and different plant extracts are some of the alternative strategies that have been explored to reduce the number of microsclerotia or wilt symptoms in multiple crops [65, 68–72]. A few studies also showed that application of beneficial bacteria significantly improved seed germination, seedling vigor, growth, yield, and early blight disease protection in tomato through multiple mechanisms including production of growth regulators and induced biosynthesis of antioxidant peroxidase and polyphenol oxidase [63]. Although many different strains of microbes belonging to multiple genera and species have been identified and tested for their efficacy, major genera of PPBs include *Bacillus*, *Paraburkholderia*, *Pseudomonas*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, and *Serratia*. Major modes of action by which PPB provide beneficial effects to host plants include production of growth promoting hormones, antibiotics, and lytic enzymes that affect harmful microbes, nitrogen fixation from the atmosphere, nutrient solubilization from soil minerals for plant availability, and systemic resistance induction in the host or treated plants. Two PPB, *Bacillus amyloliquefaciens* and *Paraburkholderia fungorum* applied on strawberry by Rahman et al. [73] not only increased yield but also significantly improved contents of several antioxidants and total antioxidant activities of fruits. Treatments of strawberry plants with bacterial strains *B. amyloliquefaciens* and *P. fungorum* consistently produced higher antioxidants, carotenoids, flavonoids, phenolics, and total anthocyanins compared to nontreated control [73]. Flores-Félix [14] reported that application of a strain of genus *Phyllobacterium* on strawberry showed significant increase in vitamin C contents in fruits.

A recent study [73] explored an environment-friendly option for boosting strawberry plant growth, fruit yield, and functional properties of fruits through the application of two plant growth promoting probiotic bacteria and compared the results with that of nontreated control. Results showed significant improvement in plant growth, yield, various antioxidant contents, and total antioxidant activities of strawberry fruits by the application of both *B. amyloliquefaciens* BCh11 and *P. fungorum* BRRh-4 treatment compared to nontreated control. Inoculation of strawberry plants separately with two bacterial isolates significantly increased vegetative growth (plant height and root length) of the strawberry plants (**Table 4**). Generally, plant growth promoting rhizobacteria facilitate plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus, and essential minerals) or modulating plant hormone levels, or indirectly by inhibiting various pathogens as biocontrol agents [11]. Early colonization of root system has the potential to preclude pathogen colonization and infection in addition to induction of disease resistance or a range of beneficial secondary metabolites. Plant height and root length also were positively influenced and varied significantly due to the plant probiotic bacterial applications. The highest plant height (20.50 cm) was observed in BRRh-4 treated plants (**Table 4**). Similar to plant height, root length also significantly ( $p < 0.05$ ) varied among the treatments and was reflected by plant vigor (**Figure 1**). A hypothetical pathway of strawberry growth, yield, and fruit quality improvement is shown in **Figure 2**. Results from this study indicated that vegetative

Treatment	Plant height (cm)	Root length (cm)	Total fruit weight/plant (g)	Total anthocyanin content	Total phenolic content	Total antioxidant activity
Control	18.6 ± 1.01a	19.3 ± 0.43b	316.6 ± 10.06b	81.1 ± 0.5b	317.1 ± 7.3b	250.9 ± 3.1b
BChi1*	119.3 ± 0.86a	22.7 ± 0.33a	453.0 ± 2.2a	187.5 ± 16.9a	377.8 ± 1.7a	382.0 ± 1.4a
BRRh-4	20.5 ± 0.26a	23.5 ± 1.15a	467.8 ± 2.2a	223.0 ± 3.6a	380.5 ± 5.1a	385.5 ± 3.4a

Cumulative fruit harvest from each plot was recorded. The required amounts of fruit tissues from first harvest were subjected to analyses for phenolics and other antioxidants mentioned in the table. Values are means ± standard errors of three independent replications (n = 3). Different superscripted letters within the column indicate statistically significant differences among the treatments according to Fisher's protected LSD (least significance difference) test at p ≤ 0.05, adapted from [73].

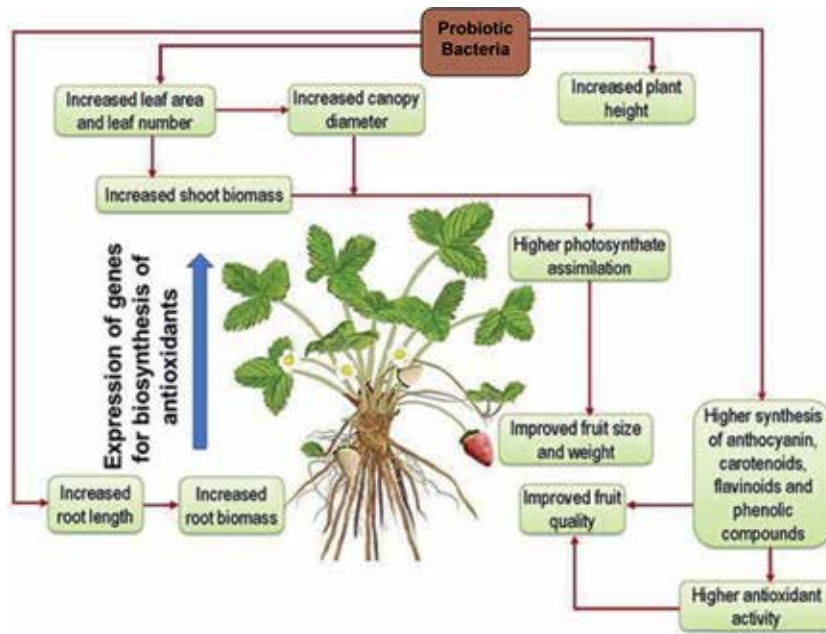
**Table 4.**  
 Effect of plant probiotic bacteria on yield and antioxidant content in strawberry fruit.

growth enhancement by probiotic bacteria may have also enhanced fruit yield and quality, enhancing secondary metabolites such as anthocyanins, phenolics, and total antioxidant activity (Table 4). Strawberry fruit from *Paraburkholderia fungorum* BRRh-4 and *Bacillus amyloliquefaciens* BChi1 treated plants had total phenolic content 380.5 and 377.72 µg gallic acid/g fruit, respectively compared with 317.08 µg gallic acid/g fruit in untreated control plants. Detailed experimental protocol can be found in a study by Rahman et al. [73].

One of the interesting findings of this study is that both plant probiotic bacteria significantly improved growth and yield of strawberry almost at the same level with some minor differences although they belong to different bacterial genera. Probiotic bacterium, BRRh-4 provided the highest fruit yield increase (48%) in plants of “Strawberry Festival” compared to nontreated control (Table 4). Treatments of strawberry plants with bacterial strains BRRh-4 and BChi1 consistently produced higher antioxidants, carotenoids, flavonoids, phenolics, and total anthocyanins compared to nontreated control [73]. A previous study showed that the members of the genus *Phyllobacterium* were good plant probiotics with the capacity of increasing fruit yield as well as quality [14]. Application of plant probiotic bacteria significantly increased total anthocyanin content in strawberry



**Figure 1.**  
 Effect of different doses of chitosan and probiotic bacteria on vegetative and reproductive growth of cv. Strawberry Festival. Adapted from [58, 73].



**Figure 2.**

*A hypothetical pathway of stimulation of fruit yield and accumulation of antioxidants in strawberry fruit due to the root colonization by probiotic bacteria.*

fruits compared to nontreated control. The highest anthocyanin content (222.0 mg cyanidin-3-*O*-glucoside/100 g fruit) in strawberry fruits was recorded in plants treated with BRRh-4 followed by BChi1 (187.47 mg cyanidin-3-*O*-glucoside/100 g fruit). To evaluate whether plant probiotic bacteria had any effect on antioxidant activities of strawberry fruits obtained from both probiotic bacteria and nontreated control plants, we estimated total antioxidant activities of fresh strawberry fruits by DPPH assay. The results of the DPPH assay for total antioxidant activity were expressed as butylated hydroxytoluene (BHT) equivalents per gram of strawberry fruit. As expected, the total antioxidant activity of fresh strawberry fruits was the highest in BRRh-4 (385.47 µg BHT/g fruit) followed by BChi1 treatment (382.00 µg BHT/g fruit) (Table 4) [73].

#### 4.1 Bio-rational/natural product-based approach for strawberry root disease management for boosting yield

The strawberry black root rot complex (BRRC) and crown rot are increasing problems in perennial strawberry plantings worldwide and have been identified as limiting factors of sustainable strawberry production [74, 75]. Yield loss from black root rot alone can range from 20 to 50% [76], which can dramatically increase if crown rot occurs concurrently. Because several factors are involved in BRRC of strawberry, including a range of infectious agents (nematodes and root infecting fungi) and various abiotic factors such as poor soil characteristics [77], the disease control is complicated, and no general control measure is completely effective. On the other hand, crown rot disease of strawberry caused primarily by the fungal species *Colletotrichum gloeosporioides* and *Phytophthora cactorum* [78] can sometimes also incur significant yield loss in strawberry production in the US and other strawberry growing countries [78]. Although inoculum sources for crown rot in fruiting fields may be diverse, infected planting stock is the



most important source of *C. gloeosporioides* [79–83] whereas *P. cactorum* is mostly soil-borne and builds up in a strawberry field over time. Occurrence of crown rot caused by *Fusarium oxysporum* f.sp. *fragariae* is also on the rise. Mass [84] observed that in many cases where crop rotation was not an option, fumigation of soil was necessary to control soil-borne diseases. Methyl bromide (MeBr) was previously used as a preplant broad-spectrum soil fumigant to control soil-borne diseases, nematodes, insects, and weeds in high value crop such as strawberry [85]. However, with the disappearance of this highly effective soil fumigant MeBr, and restrictions on the allowed use of other alternative synthetic fumigants, the interest in the development of safe, sustainable, and economically viable fumigation strategies have increased to manage soil-borne fungi and nematodes [85]. More importantly, the demands from organic growers and small growers who cannot use synthetic fumigants have increased tremendously [86]. Alternative strategies are also required especially for strawberries as disease-resistant cultivars are unavailable [87]. Among multiple alternatives of soil fumigation with synthetic chemicals, glucosinolate-containing Brassica spp. is known to release volatile isothiocyanates (ITCs), which are toxic to different pathogens [88]. The chemistry involved in the biofumigation can be attributed to the action of myrosinase enzyme on the glucosinolates (GLS) to release ITCs, thiocyanates, nitriles, oxazolidine, dimethyl sulfide, and methanethiol, among other compounds [88, 89]. Several lines of evidence suggest that biofumigation with ITC-producing plants have shown promising results against soil-borne fungal pathogens, for example, *Rhizoctonia*, *Verticillium*, *Fusarium*, *Pythium*, and *Phytophthora* spp. [90–92]. However, the concentration of ITCs produced is influenced by mustard variety [93], soil texture, moisture, temperature, microbial community, and pH [94, 95], resulting in variable soil-borne disease control efficacy. From a NE-SARE funded project in the U.S., Balzano [93] found the highest glucosinolate content and biomass in “Caliente-199.” While these observations indicate a need for selecting the right variety, site-specific testing, optimization of the method such as selection of the best growth stage (highest content of glucosinolate), optimum tissue disruption, and quick soil incorporation may also play a significant role. This is crucial for the success of this approach as laboratory experiments indicated that the efficacy of the conversion to ITCs was only 5% of the potential when using tissue disruption methods (cutting and chopping) similar to those frequently used under field conditions [95, 96]. Matthiessen et al. [97] were able to increase soil ITC levels by 20-fold (100 nmol per g soil) using a tractor-drawn tissue pulverizing implement compared to when using a cutting and chopping implement. In addition, they showed that adding excess water to the pulverized tissue was necessary for maximum ITC release.

Another promising nonchemical soil-borne disease control alternative is anaerobic soil disinfestation (ASD), which was adapted from the previously described methods of biological soil disinfestation (BSD) and soil reductive sterilization [98, 99] to create a treatment suitable for strawberry [100]. A wide range of soil-borne plant pathogens and plant parasitic nematodes have been controlled in a variety of crops using ASD [100]. Implementation of ASD is done in three different steps. First, a labile carbon source is added to the soil followed by the generation of anaerobic conditions through application of water to fill soil pore space. In the third step, the soil is covered with plastic mulch to prevent oxygen exchange. The exact mechanisms that lead to disease suppression with ASD are not clearly understood but may involve production of organic acids and other biologically active volatiles [101] and amplification of specific microbes with biocontrol activity [102].

## 5. Future perspectives

Findings from many important and relevant studies indicated that natural products and plant probiotic bacteria especially the ones isolated from the native environment could be used as natural agents for sustainable production of high quality strawberry with no or little additional use of expensive synthetic inputs. However, researchers found that the effects are more pronounced in nutrient poor growing conditions. The additive effect of utilization of these products in growing environment with balanced nutrition has not been sufficiently researched. In addition, with the disappearance of soil fumigant methyl bromide that played an essential role in managing soil-borne diseases in high value crop like strawberry, more research should be directed to finding natural alternatives for sustainable management of both foliar and soil-borne diseases. Resistance development in plant pathogens against synthetic products is also a huge concern that dictates the need for developing sustainable options. Numerous natural products and microbial strains have been screened for antimicrobial properties with positive outcomes enabling plants to resist important phytopathogens and provide plant growth-promoting effects. However, only a few of these products are commercially available to growers. Large-scale use of these products did not occur due to the variability and inconsistency of results in field conditions. Lowering the variability and increasing the consistency of results from these products are among a few challenges that will have to be addressed. The scientific community must determine the factors that interfere with the reproducibility of results from one location to another or controlled condition to field condition over time. Integration of these products with other management options should help in reducing the variability of results and produce additive effects. The continuing need for natural products supporting sustainable strawberry production will make discovery and commercialization of natural and beneficial microbe-based products as an attractive and profitable pursuit in the coming days.

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# Management of Strawberry Insect and Mite Pests in Greenhouse and Field Crops

*Oscar Liburd and Elena Rhodes*

## Abstract

Strawberry is an important small fruit crop grown throughout the world due to its rich sources of vitamins and abundance of antioxidants. The US is the world's leading producer of strawberries followed by Mexico. The main strawberry producing states are California followed by Florida, which produce >90% of the strawberries grown in the US. Strawberry production is often threatened by a host of arthropod pests that include insect and mite species. In order for the US to maintain its lead in strawberry production it is vital to develop effective management tools for key insect pests, diseases, and weeds. Some of the major arthropod pests that affect strawberry production include twospotted spider mites, *Tetranychus urticae* Koch, thrips, *Frankliniella* and *Scirtothrips* spp., armyworms, root-boring pests, and many different hemipterans that cause injury to the strawberry leaf and fruit including the tarnished plant bug, *Lygus hesperus*, and the seed bug *Neopamera bilobata* Say. This chapter will summarize some of the key pests that can severely impact strawberry production. We have included some integrated management guidelines to curtail pest's activities during a production season.

**Keywords:** arthropods, biological control, integrated pest management, monitoring, strawberry

## 1. Introduction

Strawberry is an important crop in the United States with production in 2017 valued at \$3.5 billion USD [1]. Fresh market strawberries dominate this production with a \$3.3 billion USD value. California is the largest producer of strawberries in the United States with 15,459 hectares planted to strawberries in 2017. Florida is the second largest producer with 4330 hectares. The remaining 2000 hectares is spread among Michigan, New York, North Carolina, Ohio, Oregon, Pennsylvania, Washington, and Wisconsin. Pest management is a crucial part of strawberry production especially when even cosmetic injury can make fresh fruit unmarketable.

There are a number of insect and mite pests that can have detrimental effects on strawberry production. Some pests, such as the twospotted spider mite, *Tetranychus urticae* Koch, are major pests wherever strawberry plants are grown. Others are major pests in certain regions or under specific production systems. Becoming familiar with the mites and insects that are likely to cause economic damage in production systems is an important first step in an integrated pest management (IPM) program [2].

Monitoring is a critical component of a successful IPM program [2]. Scouting is the most common monitoring technique used in strawberry production in the United States [3]. Scouting involves examining a sample of strawberry plants from the field or greenhouse for the presence and abundance of pest mites and insects. A sampling plan should be designed to get a good representation of what is happening in the field or greenhouse. There are also monitoring traps available for certain pest insects. Yellow sticky traps, for example, are often used to monitor for aphids and whiteflies in field and greenhouse situations. The information gained from monitoring is used to determine if a treatment action, such as an insecticide application, is warranted.

Action thresholds are needed to determine when a treatment is warranted [2]. An action threshold is the point where the cost of control is less than the economic damage that will result if the pest is left untreated. These vary depending on the pest, region, production system, etc. Numbers of natural enemies present in the field or greenhouse should also be considered when deciding if a treatment action is necessary. The action threshold for releasing biological control agents, such as predatory mites, will be different than the action threshold for an insecticide or miticide application.

Strawberries can be produced in the open field and in greenhouse settings. In the United States, there are two major field production systems for strawberry production [3]. In warmer, southern areas, such as Florida and southern California, strawberries are grown as an annual crop on raised beds covered with plastic mulch. The production season is lengthy (October through March in Florida with berries harvested December through March). In contrast, strawberries are grown as a perennial crop in northern areas of the United States. Matted rows are used and the harvest season is short, occurring during the summer months. Recently, researchers and growers have been experimenting with using high and low tunnel systems. These systems use tunnels to extend the growing season in colder areas. The pest complex in each system (tunnel, greenhouse and field) overlaps but is usually different.

This chapter will discuss the pest complex of each production system. Descriptions of pests, the injury they cause, and management strategies will be presented. Management strategies will include monitoring methods, action thresholds, and treatment options.

## **2. Pest complexes of strawberry production systems in the United States**

### **2.1 Greenhouse production**

A well-constructed and maintained greenhouse can prevent larger insects, like moths, from accessing the plants inside. For this reason, the major pests of greenhouse grown strawberries are the twospotted spider mite, aphids, whiteflies, and thrips. Other strawberry pests can, however, be introduced into the greenhouse on infested transplants and equipment.

### **2.2 Annual field production**

Key pests in annual strawberry production throughout its range include the twospotted spider mite and spotted wing drosophila (SWD), *Drosophila suzukii* Matsumura. Other potential pests include cyclamen mites, aphids, whiteflies, spittlebugs, flower thrips, chili thrips, armyworms and related Noctuid caterpillars, strawberry leafrollers, tarnished plant bugs, strawberry seed bugs, and sap beetles.

### 2.3 Perennial field production

The two key pests (twospotted spider mite and spotted wing drosophila) in annual strawberry production are also key pests in perennial production systems. There are also several beetles that can be major pests in perennial strawberries, which are not present in annual systems. Other potential pests, in addition to those listed under annual field production, include potato leafhoppers and cutworms.

### 2.4 High and low tunnel systems

The pest complex in a high or low tunnel system will be similar to the pest complex in field grown strawberries in the same region. The warmer temperatures in the tunnels may lead to increased pest outbreaks in tunnels compared with field strawberries.

## 3. Leaf pests

Leaf pests can be divided into two main groups, sucking pests and chewing pests. Sucking pests include twospotted spider mites, cyclamen mites, chili thrips, aphids, whiteflies, potato leaf hoppers, and spittlebugs. Chewing pests include armyworms, cutworms, and strawberry leafrollers. Injury to leaves reduces the plants ability to photosynthesize (make food), which can reduce the quality and quantity of fruit produced. In perennial production, this injury can affect the yield the following season, also [3].

### 3.1 Twospotted spider mite

Twospotted spider mites, *Tetranychus urticae* Koch (**Figure 1**) are oval shaped and tiny. The adults are 0.5 mm in length, which is about the size of a period in 12-point font. They are usually light greenish-yellow in color with two large, dark spots on their abdomens. However, brown, red, orange, and darker green forms also occur. The eggs are spherical and clear to tan in color. The eggs and all stages of mites are usually found on the undersides of leaves. The twospotted spider mite life cycle progresses through five stages: egg, six-legged larvae, protonymph, deutonymph, and adult. Each of the three intermediate stages feeds and grows for



**Figure 1.** Twospotted spider mites and eggs on the underside of a strawberry leaf as seen under a microscope (photo credit: L. Buss, UF).

only a short time before entering a resting state prior to molting to the next stage [4]. Females are larger and rounder than males. Development from egg to mature adult takes an average of 19 days, although this time can be as short as 5 days [4]. Optimal conditions for development are high temperatures (up to 38°C) and low humidity [5, 6].

During feeding, twospotted spider mites puncture leaf cells and suck out their contents [3]. The feeding injury causes affected leaves to have a stippled appearance (**Figure 2a and b**). Spider mites do produce webbing, which is where their common name originates from (**Figure 2c**). The webbing provides protection from predators and may also help maintain favorable temperature and humidity conditions for the mites. Webbing is only visible when populations are high and, therefore, economic damage to the crop is already occurring.

Monitoring can be accomplished by checking and counting twospotted spider mites on the underside of strawberry leaves with a 10x hand lens [3]. Alternatively, strawberry leaves can be collected from the field and examined later. Leaf samples should be processed within 3 days of collection if possible. In annual production, sampling for twospotted spider mites should begin after the transplants have become established. In perennial culture, sampling should start once the new leaves fully open in the Spring. Sample 13–25 fully mature leaves per hectare. Counting the number of spider mites on each leaf will give more accurate results, but it can be time consuming. An alternative is simply to note how many leaves have spider mites present on them. A presence/absence system is used



**Figure 2.** Examples of (a) and (b) stippling injury to strawberry leaves and (c) spider mite webbing (photo credits: (a) and (b) T.W. Nyoike, UF; and (c) E.M. Rhodes, UF).

sometimes on large fields but this assessment method has some limitations and a history of mite population in the field is sometimes needed to improve its accuracy. A common threshold for this type of monitoring is 5% of leaves infested. In California, the treatment threshold is an average of 5 mites per leaf before the harvest period begins, which increases to an average of 15–20 mites per leaf once the harvest period begins [7]. An average of 10 spider mites per leaf is a common threshold used in Florida production [8].

There are a number of miticides available for twospotted spider mite management. Thorough spray coverage is essential for effective applications [3]. Rotating modes of action is also very important as spider mites can quickly develop resistance to miticides due to their short life cycle.

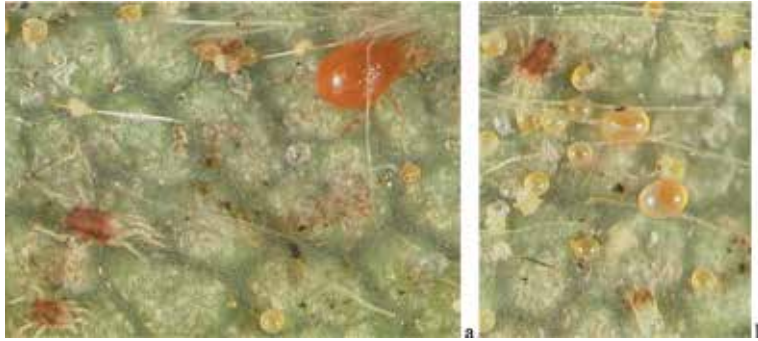
There are several commercially available predatory mite species that can be used in a twospotted spider mite management program [3]. They are especially effective in greenhouse settings. *Neoseiulus californicus* (McGregor) and *Phytoseiulus persimilis* Athias-Henriot are two commonly used species [9, 10]. *Neoseiulus californicus* mites (**Figure 3**) are orange to peach in color and slightly larger than twospotted spider mites. Their eggs are milky white and ovoid in shape. They prefer spider mites as prey and can subsist on other food sources, such as small insects and pollen, when twospotted spider mite numbers are low. For this reason, preventative releases of small numbers of *N. californicus* early in the season can reduce the severity of mite outbreaks and even eliminate them all together in some cases. In contrast, *P. persimilis* mites (**Figure 4**) specialize on spider mites, so their population declines rapidly once the spider mite population has declined to low levels. This can necessitate multiple releases in a season. Adults are pink in color with spider-like legs. Eggs are similar in shape to *N. californicus* eggs and have a pinkish to orangish tint. Preventative and curative rates are listed on supplier websites. If spider mite numbers are high, it is advisable to knock down the population with a miticide before predatory mites are released. Predatory mites should be released 7–10 days after the miticide application.

### 3.2 Cyclamen mite

Cyclamen mites, *Phytonemus pallidus* (Banks), are half the size of spider mites, ~0.25 mm, and invisible to the naked eye [3, 7]. Adults are shiny and pinkish to orangish in color while immatures are opaque white. Eggs are ovoid and translucent. They feed on developing tissues and are most commonly found on unfolded



**Figure 3.** *N. californicus* mite and eggs on a strawberry leaf (photo credit: E.M. Rhodes, UF).



**Figure 4.** *P. persimilis* (a) adult and (b) eggs with twospotted spider mites and eggs on a strawberry leaf (photo credit: E. M. Rhodes, UF).

leaves near the midvein and in flower buds. Infested leaves are small and wrinkled. High infestations can cause a compact mass of these leaves in the center of plants. Injury to leaves causes them to turn brown, wither, and die. Any fruit produced is stunted and russeted.

Cyclamen mites can persist in perennial production systems, making them a greater threat to these systems [3]. They often come into a field on infested transplants and can also be transported by insects, birds, and even people via contaminated clothes or equipment. The threshold is low, 1 mite per 10 leaves in California [7]. Miticides must be applied in large volumes of water, 2839–4732 liters per hectare, to ensure soaking of the developing tissues in the crown.

### 3.3 Chili thrips

Chili thrips, *Scirtothrips dorsalis* Hood, is a more recently introduced pest that was first reported in the US from Florida in 2004 and Texas in 2008 [11]. It has become a serious pest of multiple crops and has the potential to spread throughout the southern United States and in western states such as California [11]. Chili thrips adults (**Figure 5**) are tiny, only 1.2 mm in length [12, 13]. They are pale yellow in color except for their dark wings, which are bristle-like, and dark spots along the top of the abdomen that form an incomplete stripe. Immatures are pale yellow in color and lack wings.

Adults and immatures feed on young strawberry leaves causing darkening of the leaf veins near the leaf base [12, 13]. As the infestation progresses, the darkening



**Figure 5.** *Chili thrips* adult (photo credit: B. Panthi, UF).



becomes streaks on the leaves that can cover the entire leaf surface and lead to leaf deformation. Heavy infestations can cause stunting of entire plants. Infestations early in the season when strawberry plants are small and have only a few leaves cause the most damage.

Scouting for Chili thrips is best accomplished by looking for the injury they cause [12]. Leaves with the characteristic injury described above can be gently tapped over a white sheet of paper. This will dislodge any Chili thrips present, which will resemble tiny, yellow dashes crawling on the piece of paper. Thresholds have not yet been developed.

The predatory mite *Amblyseius swirskii* (Athias-Henriot) has shown some efficacy against thrips species in vegetable systems [14]. These predators feed primarily on eggs and immatures of thrips. This predatory mite is commercially available and has the potential to be used in other crops like strawberry. Insecticides should be avoided when possible because they cause disruption in the predator complex and cause thrips population to flare up. Insecticides should only be applied if the infestation is severe, particularly if thrips numbers are high early in the season.

### 3.4 Aphids

There are four species of aphids (**Figure 6**) commonly found in strawberries: the green peach aphid, *Myzus persicae* (Sulzer), the melon aphid, *Aphis gossypii* Glover, the potato aphid, *Macrosiphum euphorbiae* (Thomas), and the strawberry aphid, *Chaetosiphon fragaefolii* (Cockerell) [3, 7]. The strawberry aphid can severely affect yield because it transmits a host of virus including the strawberry mild yellow edge virus, strawberry crinkle virus and the strawberry mottle virus [7, 15]. In general, aphids are small (2.54 cm), globular, pear-shaped, soft-bodied insects found on the underside of leaves. Aphids are often various shades of green or yellow but can be black or even pink. There are winged and wingless forms. Aphids can be distinguished from similar insects by the two cornicles (tube-like structures) protruding from the end of the abdomen.

Winged females enter fields from nearby infested crops or weedy areas [3, 7]. They can also enter greenhouses. Female aphids can produce daughters without mating, so populations can build up very quickly. Aphids use their sucking mouthparts to feed on plant juices and excrete excess sugar as a sticky, sugary honeydew. Sooty mold fungus will grow on the honeydew and can contaminate fruit. Aphids efficiently transmit plant viruses. In strawberry production, these viruses are mainly a concern in nursery production. Viruses can also persist from one season to another in perennial production, but this is a rare occurrence.



**Figure 6.**  
*Aphids on a grape leaf (photo credit: B. Achhami, UF).*

Aphids rarely reach damaging levels in field grown strawberries [3]. Monitoring for aphids involves examining leaves in the field or collecting the leaves and examining them elsewhere. In Southern California, where they can be as issue, the threshold is 30% infested leaves from a sample of 100 leaves per hectare [7]. Yellow sticky traps can be used to monitor winged aphid populations and are especially useful in greenhouses.

Aphids have many natural enemies and it is important to take parasitism into consideration when deciding if a spray is necessary. Parasitized aphids, often called mummies, are swollen, brown, and sometimes have a hole chewed in the abdomen if the adult parasitoid wasp has emerged [3, 7]. The adult parasitoids are tiny wasps that lay eggs in aphids. The larvae develop inside the aphids, feeding on the aphid from the inside out and killing it in the process. The larva pupates in the aphid mummy and the adult wasp chews a hole in the aphid to emerge and begin the cycle again. Several aphid parasitoids are available commercially and are particularly effective in greenhouses. Green lacewing larvae, *Chrysoperla rufilabris* (Burmeister) and other generalist predators will also prey on aphids.

### 3.5 Whiteflies

There are many species of whiteflies (**Figure 7**) that can be found in strawberry [3, 7]. These include the bandedwing whitefly, *Trialeurodes abutilonia* (Hold.), greenhouse whitefly, *T. vaporariorum* (West.), iris whitefly, *Aleyrodes spiroeoides* Quaintance, silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring, and the strawberry whitefly, *T. packardi* (Morrill). Whitefly adults are yellowish in color with white wings. Immatures, called nymphs, resemble scale insects. The first immature stage is a crawler that moves a short distance from the eggs before becoming sedentary and forming its protective scale. The final nymphal stage is nonfeeding and often called a pupal stage. All stages are found on the underside of leaves. Adults and nymphs suck plant juices and produce honeydew like aphids. Whiteflies can also vector some viruses.

Whiteflies are typically not an important pest in strawberry but can be more of a nuisance pest [3]. They are of concern in greenhouse strawberry production and in field production in California [7]. In California, the greenhouse whitefly vectors pallidosis-related decline of strawberry [7].

Adult whiteflies can be monitored using yellow sticky traps [7]. One trap per 25 hectares should be hung from a stake just above the crop canopy and checked weekly. It is important to monitor nymphs at the same time. This is done by checking 20 mature leaves from each field quarter. There is no treatment threshold.



**Figure 7.**  
Whiteflies on a squash leaf (photo credit: D. frank, UF).

In California, it is recommended to treat when the population appears to be increasing at a rapid rate and parasitism levels are low [7].

Whitefly nymphs are parasitized by tiny wasps, like aphids, and parasitized nymphs are black in color [3, 7]. Some whitefly parasitoids including *Encarsia formosa* and *Eretmocerus* spp. are available commercially and generalist predators, *Delphastus* spp. feed on them as well. Thorough coverage is essential when applying insecticides.

### 3.6 Potato leafhopper

Potato leafhoppers, *Empoasca fabae* Harris, are bright green leafhoppers [3, 16]. Nymphs are lighter green in color and wingless. The nymphs will move sideways when disturbed, which distinguishes them from other leafhoppers. Potato leafhoppers are larger than whiteflies and aphids, with adults being about 8.5 mm long. They overwinter in southern states with mild winters and migrate north as the weather warms up. They reach as far north as Canada. They generally reach North Carolina by early summer and continue north from there [16]. They feed and reproduce throughout the summer and fall until they are killed off by the first frost. Like the other sucking pests, potato leafhoppers are found on the underside of leaves.

Potato leafhoppers feed by sucking out plant juices. They prefer to feed on new growth. Unlike other sucking pests, potato leafhoppers inject toxins into the plant tissue with their saliva [3, 16]. These toxins cause stunting, curling, and browning of leaves. This is often called “hopperburn” and resembles herbicide burn. In perennial strawberries, high amounts of hopperburn can cause reduced growth and yield in the next season. There are no established thresholds, but insecticides should be applied before hopperburn becomes widespread.

### 3.7 Other sucking pests

Many other mites and sucking insects are encountered in strawberry plantings. Most are encountered occasionally in small numbers. However, there is the potential for some to become pests. Spittlebugs, mealybugs, and stink bugs are examples of these.

Spittlebugs, Cercopidae, are immature froghoppers. Adult froghoppers resemble leafhoppers but are usually larger and flatter than leafhoppers [17]. The nymphs secrete a frothy foam for protection (**Figure 8**) that resembles spittle, hence their common name. The species *Philaenus spumarius* (L.) can be a pest in strawberries, primarily because the foam can be annoying to pickers [3].

Mealybugs, Pseudococcidae, are covered in waxy or mealy secretions for protection [17]. Adults are small and oval in shape with well-developed legs. Currently, there are no major mealybug pests of strawberries, but several species are serious pests of other crops and ornamentals. However, mealybugs are sometimes seen in strawberries, so it is important to be aware of them [3].

Stink bugs, Pentatomidae, are a family of true bugs that are shield shaped and secrete foul-smelling chemicals when disturbed [17]. Many species are various shades of green or brown. Other species, like the harlequin bug, *Murgantia histrionica* (Hahn), sport very bright colors. Some stink bug species, like the harlequin bug and southern green stink bug, *Nezara viridula* (L.), are major pest species [17]. Some of these pest species can be found in strawberries but rarely build up to damaging numbers. Other species of stink bug are predatory, like the well-known spined soldier bug, *Podisus maculiventris* (Say), and feed on other insects [17]. It is



**Figure 8.** Spittlebug protective frothy foam. The spittle bug nymphs are underneath the foam and cannot be seen (photo credit: E.M. Rhodes, UF).

important to properly identify any stink bug causing concern in strawberries to make sure it is not a beneficial predatory species.

### 3.8 Caterpillars

Armyworms, cutworms, the strawberry leafroller, and saltmarsh caterpillars all feed on strawberry leaves and sometimes chew holes in fruit [3]. Cutworms will also feed on strawberry flowers. While armyworms, cutworms, and strawberry leafrollers are considered major strawberry pests, saltmarsh caterpillars rarely reach damaging levels.

Armyworms, Noctuidae, are the caterpillars of brown, night flying moths [3, 7, 8, 17]. Eggs are laid in clusters and covered with scales from the female moth that laid them. Early instar larvae are light green with black head capsules and cluster together. The larvae become darker and develop white, vertical stripes that travel the length of the body as they mature (**Figure 9**). Armyworm larvae feed on the undersides of leaves. Early instar larvae leave the top layers of the leaf tissue intact while larger, later instar larvae consume all the leaf tissue and can quickly skeletonize leaves. Leaf injury can lead to a decrease in the quality and quantity of fruit produced. Armyworms can also cause direct injury by feeding on fruit. The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) and southern armyworm, *Spodoptera eridania* (Stoll) are the species most commonly found on strawberries in Florida [8], whereas the beet armyworm, *Spodoptera exigua* (Hübner), is the main armyworm found in strawberries in California [7]. In California, beet armyworm adults are monitored using pheromone traps put out just prior to transplanting. If trap catches are high, the young strawberry plants should be examined for egg masses. It is important to time insecticide applications after egg hatch when larvae are early instars.

Cutworms are also the caterpillars of Noctuid moths [3, 17]. They can be a major pest in perennial strawberry systems. The caterpillars are smooth, like armyworms, and mottled brown or gray in color. The black cutworm, *Agrotis ipsilon* (Hufnagel), is the most common species causing damage in California [7]. Cutworms chew holes in leaves and cut stems. It is this stem cutting that gives them their common name. They will sometimes feed on flowers, which cause fruit to be deformed and unmarketable. Injury often occurs along field margins, so monitoring should focus



**Figure 9.**  
*Later instar fall armyworm on velvet bean plant (photo credit: C. Scott, UF).*

on these areas [3, 7]. As with armyworms, young plants are especially vulnerable. There are no established thresholds.

The strawberry leaf roller, *Ancylis comptana* (Frölich) is an occasional pest of strawberries in the United States [3, 18]. The adults are small, reddish-brown moths (**Figure 10a**). Early instar caterpillars are pale green with brown heads (**Figure 10b**) while later instar larvae are gray-brown with yellowish-brown heads. Early instar larvae feed on the underside of strawberry leaves near veins. They produce a silky covering for protection. They grow and eventually move to the top of the leaf and fold or roll leaf edges together. The rolled leaves protect them from predators and environmental factors. The caterpillars pupate in the leaf roll. Strawberries can tolerate 10–20% of their leaves being infested with leafrollers, so management is often unnecessary [3, 18]. Removal and destruction of infested leaves can prevent infestations from building up. If a spray is necessary, thorough coverage is essential.

Salt marsh caterpillars (**Figure 11**), *Estigmene acrea* (Drury), are the caterpillars of a species of tiger moth [17]. The adult moths are white with black spots with a pinkish to orangish abdomen that also has black spots. Female hind wings are white while male hind wings are yellowish to orangish in color. The caterpillars are



**Figure 10.**  
*Strawberry leaf roller (a) adult and (b) caterpillar (photo credit J. Renkema, UF).*



**Figure 11.**  
*Later instar salt marsh caterpillar (photo credit: M. Lopez, UF).*

covered in hairs that can be white, various shades of yellow and orange, and black. The early instars cluster together to feed and then disperse as they grow. Mature caterpillars can be 5 cm long and look even larger because of their hairs. Salt marsh caterpillars prefer grasses and rarely occur in large enough numbers in strawberry plantings to be of concern.

### 3.9 Other chewing pests

It is possible for other caterpillars to be seen feeding on strawberry. The lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller), a serious pest of corn, is occasionally seen in strawberries, for example [3]. Various species of crickets and grasshoppers will feed on strawberry leaves, though they rarely occur in large numbers or cause enough injury to be of concern. The same is true of some species of earwigs.

## 4. Root pests

Root pests are only a concern in perennial strawberry production systems [3]. The strawberry root worm, strawberry root weevil, black vine weevil, and white grubs all attack roots. These are all beetles and it is the immatures, or grubs, that feed on the roots. Injury to roots reduces the plants ability to draw water and nutrients from the soil, which can lead to reduced plant vigor, yield, and even plant death. The injury can also provide an entry point for diseases. Adults feed on leaves and can, occasionally, reach damaging levels.

### 4.1 Weevils

The most damaging of the root pests are the weevils. Weevils are easily distinguished from other beetles by the snout-like structure that protrudes from the front of their heads [17]. Strawberry root weevils, *Otiorhynchus ovatus* (L.), adults are approximately 6.4 mm long and brown to black in color [3, 7]. Black vine weevils,

*O. sulcatus* Fabricius, are black flecked with yellow and larger at approximately 1.27 cm long [3, 7]. Adults of both species emerge in late spring and early summer and feed on leaves at night while sheltering near the base of plants during the daytime [3, 7]. They feed on the leaf margins, producing distinctive hook-shaped notches. Adults lay eggs in the fall and larvae feed on roots before burrowing deeper into the soil to overwinter. The larvae are legless, crescent shaped, creamy white to light pink grubs similar in size to the adults. As temperatures warm up in the spring, larvae again feed on roots before burrowing into plant crowns to pupate. Infested plants are dull and reddish, wilted, stunted, and extremely susceptible to stress. Injury in a field often occurs in a circular pattern. Adults emerge from these pupae to begin the cycle again.

Managing these weevil pests is difficult. Sanitizing farm equipment is important to avoid moving beetles from infested fields into fields that are not infested. Heavily infested fields should be plowed to prevent the spread of the weevil and repeated disking will continue to expose grubs to predation and weather. Soil fumigation after plowing is also effective. Two grubs per plant can cause economic damage, which makes for a low threshold [3, 7]. There are insecticides available to reduce the population of adults before they lay eggs, but efficacy is limited. Using nematodes that parasitize grubs in combination with insecticides to manage adults has shown some success. *Steinernema feltiae* (Filipjev) and *Heterorhabditis bacteriophora* (Poinar) are both commercially available and can be used in combination. Soil should be moist when nematodes are released.

#### 4.2 Strawberry rootworm

The strawberry rootworm, *Paria fragaria* Wilcox, is the smallest of these beetle pests [3, 19]. Both adults and grubs are only 3.2 mm long. Adults are shiny and vary from brown in color with four black splotches to completely black. They chew holes in leaves and can skeletonize leaves when infestations are severe. Adults overwinter in sheltered places such as ground litter and become active as temperatures warm in the Spring. In the northern United States, high numbers can occur in May and June, though this is uncommon [19]. These adults lay eggs in the soil that hatch into grubs, which are milky white in color and have six legs. Larvae feed on roots, which can cause plants to be stunted, and then pupate in the soil. Adults emerge from these pupae in July and August. This second generation of adults will overwinter and begin the cycle again. Insecticide applications should be timed when adults are feeding [3, 19].

#### 4.3 White grubs

White grubs, which are the larvae of Scarab beetles such as the June Beetle, Japanese Beetle, and Rose Chafer, can also be an issue in perennial strawberries [3, 20]. The grubs are C-shaped, milky white in color, have six legs, and vary in size depending on species. White grubs can grow as large as 2.5–3.8 cm long. They are a major pest of sod and can move into strawberry fields from adjacent grassy areas [3, 20]. June beetles lay their eggs in early summer. The larvae feed on roots and burrow deeper in the soil to overwinter. Larvae repeat this pattern for the next two seasons, pupating in the soil at the end of the 3rd summer. Most white grub adults have a similar 3-year life cycle [3, 20]. However, a few, like the Japanese beetle, have a 1-year life cycle. Fields planted to sod should be left fallow or planted to non-susceptible hosts, such as squash and its relatives, for several seasons before strawberries are planted there [3, 20]. Prevention is key because grubs are difficult to control once they infest a field.

## 5. Flower pests

Strawberry bud weevils feed on and lay their eggs in strawberry buds and can cause significant yield losses. Flower thrips feed in open flowers. Tarnished plant bugs feed on both flowers and fruit. Injury to flowers can result in deformed fruit that is unmarketable. Severely injured buds and flowers may not develop into fruit. Both situations cause yield loss.

### 5.1 Strawberry bud weevil

The strawberry bud weevil, *Anthonomus signatus* (Say), also known as the strawberry clipper, is a highly destructive pest in perennial strawberries [3, 21]. Adult weevils are only 1/10"–1/8" long and are copper colored with two large, black spots on the back of their wing covers. Adults become active in the Spring when temperatures reach about 60°F. Adult females chew a hole in a developing flower bud and use their long snout to feed on the pollen. They then lay a single egg in the bud and girdle or clip the pedicel, which is the stem that attaches the bud to the plant, right at the base of the bud. The buds turn brown and eventually fall to the ground. The small, opaque white, legless grub develops and then pupates in the bud. Adults emerge in June, feed on flower pollen, and then seek overwintering sites, where they stay until the following Spring. Preferred overwintering sites include mulches, brush, woods, and woodlots.

Girdled buds do not become fruit, so strawberry bud weevils can cause substantial yield loss if populations are high. Monitoring should begin once strawberry flower buds appear in the spring [3, 21]. In smaller fields, sample two feet of row from 5–10 areas of the field counting the number of cut buds in each section. For larger fields, sample five 10-foot sections of row. The threshold is one cut bud per foot of row. Two applications of insecticides should be made if the threshold is met or exceeded, one right away and the 2nd 10 days after the first.

Reducing overwintering sites for adult weevils is an important part of managing this pest [3, 21]. This includes plowing under old beds as soon after the end of harvest as possible and removing dead leaves and mulch from the field. Rotating a strawberry field to another crop after 3 years is also important in reducing the incidence of strawberry bud weevil injury and damage.

### 5.2 Flower thrips

A mix of thrips species can be found in strawberry flowers. The western flower thrips (**Figure 12**), *Frankliniella occidentalis* (Pergande), feeds on strawberry flowers along with other species, primarily in the genera *Frankliniella* and *Thrips* [3, 7, 8]. They are similar in size and appearance to chili thrips. They vary in color from light yellow to dark brown, depending on species, and lack the abdominal spots present on chili thrips. Adults are blown into strawberry fields from other hosts on wind currents. Some species lay eggs in the strawberry flowers while others simply feed on the flowers. Immature thrips are smaller than adults and lack wings. Populations of flower thrips usually migrate into strawberries when flowering resources are unavailable on other host crops or weeds. In the US, the most abundant flower thrips species in the strawberry growing region is the western flower thrips, *Frankliniella occidentalis* (Pergande) [3, 7]. Florida has a unique flower thrips species, *Frankliniella bispinosa* Morgan that is common to that region [8]. Regardless, flower thrips rarely cause economic damage in strawberry. Predatory bugs





**Figure 12.**  
*Western flower thrips female (left) and male (right) (photo credit: E.M. Rhodes, UF).*

(*Orius* spp. and *Geocoris* spp.), that are common in the strawberry system, regulate thrips populations.

Thrips possess a single mandible that they use to puncture plant tissue. The rest of the mouthparts are formed into a tube-like structure they use to suck up the contents of punctured tissues. Flower thrips feed on the ovaries in most flowers, which is the part that develops into fruit. In the case of strawberries, flower thrips feed on the expanding receptacle [3, 7]. Feeding injury can cause russeting or bronzing of strawberry fruit around the fruit cap. Dulling of the fruit and fruit cracking has also been attributed to thrips feeding injury.

Monitoring for flower thrips is done by gently tapping flowers over a white piece of paper [3, 7, 8]. The thrips will resemble tiny yellow and brown dashes crawling around on the paper. Strawberries can tolerate high numbers of flower thrips, so the treatment threshold is 10 thrips per flower [3, 7]. Minute pirate bugs, *Orius* spp., feed on flower thrips and are commercially available although release rates in strawberries have not been determined.

## 6. Fruit pests

SWD larvae, tarnished plant bugs, strawberry seed bugs, and sap beetles feed directly on strawberry fruit. SWD, tarnished plant bugs, and seed bugs can cause significant yield losses because they feed on developing and ripe fruit. Sap beetles, in contrast, prefer overripe fruit, but will chew holes in ripe fruit, which can reduce yield.

### 6.1 Spotted wing drosophila

The spotted wing drosophila, *Drosophila suzukii* Matsumura, is a more recently introduced pest of strawberries and other thin-skinned fruits. It was first found in Santa Cruz County, California in 2008 and has since spread across the United States [22, 23] Males can be distinguished from other drosophilid species by a spot present

on each wing (**Figure 13a**) and two pairs of combs on each foreleg that have four to six teeth each (**Figure 13b**) [22]. Newly emerged males lack the wing spots but can be identified by the combs. Unlike most other drosophilid species, female *D. suzukii* possess a heavily sclerotized serrated ovipositor (**Figure 13c and d**) that allows them to lay their eggs in ripening and ripe fruit [22].

Spotted wing drosophila larvae develop in the fruit, consuming the fruit as they do so, which renders the fruit unmarketable [22, 23]. As the larvae feed, areas where they are feeding will turn brown and become soft. Sunken areas that leak juice will appear on the surface of fruit. A single larva found in a shipment of fruit can cause the entire shipment to be rejected. The injury can also make the fruit susceptible to attack by other drosophilid species and diseases.

Adult spotted wing drosophila can be monitored with traps [24]. There are several commercially available traps and baits but a bait specific to spotted wing drosophila has not yet been developed. Soapy water is used as the drowning solution in these traps. Homemade traps can provide a more cost-effective option. Any plastic container with a lid that is around the size of a peanut butter jar can be used in trap construction. Punch two or three rows of holes around the middle of the plastic container leaving a 1" unpunched area so the trap contents can easily be poured out. The holes should be large enough to allow spotted wing drosophila to enter but not so large that bees and other pollinators can enter. String or a long twist tie can be used to create a hanger for the trap by tying it through holes on either side of the trap. An example of a home-made trap is shown in **Figure 14**. The most effective homemade bait is a mixture of yeast, sugar, and water. Mix 2 tsp. sugar and ¼ tsp. active dry yeast in 2/3 cup water per trap. In this case, the bait also serves as the drowning solution. Traps should be checked weekly. The contents can be



**Figure 13.** Spotted wing drosophila (a) male, (b) enlarged view of male foreleg showing the two combs, (c) female, and (d) enlarged picture of female ovipositor (photo credits: L.E. Iglesias, UF).



**Figure 14.**  
Home-made spotted wing drosophila trap baited with yeast, sugar, and water mix (photo credit: L. Iglesias, UF).

emptied into another container or dumped through a filter screen. As females often arrive earlier than males, examining the flies on the screen with a hand lens even if no flies with wing spots are present is very important. There is no threshold for spotted wing drosophila and most growers begin a spray program once flies are found in traps.

The only way to sample for larvae is by collecting fruit and placing them in the freezer. Larvae will migrate to the surface of fruit as they freeze [24]. Because strawberry fruit are large, juicy, and contain a lot of flesh relative to other berries, dissection and extraction (salt and sugar) techniques that work well with other berries are difficult to perform and do not work well. The presence of a larvae indicates the need for insecticide application.

## 6.2 Tarnished plant bugs

Two lygus bugs are known as tarnished plant bugs, *Lygus lineolaris* (palisot de Beauvois) and *L. hesperus* Knight in the eastern and western United States, respectively [3, 7, 25]. They overwinter as adults and females begin laying eggs in the spring. Early instar tarnished plant bugs are small, bright green, and resemble aphids. They can be distinguished from aphids because they move much faster than aphids and lack cornicles on their abdomens. Nymphs develop brown markings as they mature. Adults are ¼" long and bronze/yellow or mottled brown in color. Adults resemble big-eyed bugs, *Geocoris* spp., which are beneficial predatory insects. As their name suggests, big eyed bugs have much larger eyes than tarnished plant bugs.

Both nymphs and adults feed on developing flowers and fruit [3, 7, 25]. They feed on the seeds, which stops the area around the seeds from developing, which, in turn, causes fruit to be misshapen or "cat-faced." "Cat-faced" fruit are deformed, small, and their ends are seedy. Tarnished plant bugs are a serious pest in perennial strawberries and can cause 90% yield loss if not controlled. The overwintering adults and first generation of nymphs are the most destructive to perennial

strawberries because they feed during the bloom period. It is important to note that poor pollination can also cause misshapen fruit. However, fruit misshapen from poor pollination will have seeds that vary in size while those injured by tarnished plant bugs will have seeds that are uniform in size [25].

Monitoring for tarnished plant bug should begin right before the start of bloom. Thirty flower clusters should be sampled evenly across the field by gently tapping them over a white piece of paper or another white surface. Nymphs will fall onto the white paper. The threshold is 0.25 nymphs before 10% bloom or more than 4 clusters infested [3, 7, 25]. The threshold rises to 0.5 nymphs per flower from mid to late bloom. There are also devices available to vacuum tarnished plant bugs off strawberry and other plants. The threshold for vacuuming is one bug per 10 plants [7].

Controlling weeds is an important part of managing tarnished plant bugs because tarnished plant bugs feed on many different weeds [3, 7, 25]. In California, the parasitoid wasp *Anaphes iole* Girault, which is an egg parasitoid, is available commercially [7]. Bug-vacs can be used to manage mild to moderate infestations but may also remove predatory insects and spiders from the plants [7].

### 6.3 Seed bugs

The strawberry seed bug (**Figure 15**), *Neopamera bilobata* (Say), has recently become a pest of annual strawberry production systems in Florida. Adults are similar in appearance to tarnished plant bugs but are smaller and more elongated in shape. Immatures closely resemble ants, especially at a distance. Ants have elbowed antenna and a narrow “waist” (narrow constriction between thorax and abdomen) while seed bug nymphs have straight antennae and the abdomen more broadly connected to the thorax. Adults and nymphs feed on developing fruit, which is suspected to cause injury to the fruit that can make it unmarketable. Research into economic impacts, thresholds, and treatment options is in the early stages.

### 6.4 Sap beetles

The strawberry sap beetle, *Stelidota geminate* (Say), is one of many sap beetles, family Nitidulidae, that are found in strawberry plantings. Along with the strawberry sap beetle, the dusky sap beetle, *Carpophilus lugubris* Murray, and the fourspotted sap beetle, *Glischrochilus quadrisignatus* (Say), are the most common



**Figure 15.**  
*The strawberry seedbug (photo credit: O. Dosunmu).*

species found in North Carolina [26]. In contrast, *C. fumatus* Boheman, *Lobiopa insularis* (Castelnau), and *Epuraea luteola* Erichson are the most common species found in Florida strawberries [27]. In general, sap beetles are small, 1/8"–1/4" in length, and orange to dark brown in color. Several abdominal segments protrude beyond the elytra and they have clubbed antennae.

Adults fly into strawberry fields from wooded areas or other protected sites after overwintering [26]. In Florida, they do not overwinter and can come into fields at anytime but are more common in February through the end of harvest in March or April [27]. Females lay their eggs on or near overripe and rotting fruit. The larvae feed on and develop inside the fruit and then pupate in the soil nearby. The adults feed on ripe fruit, chewing small holes in the fruit that can make fruit unmarketable and introduce disease organisms.

The best way to manage sap beetles is to practice good field sanitation [26, 27]. Frequent harvests that include the removal and disposal of overripe and other unmarketable fruits usually prevent sap beetles from becoming a problem.

## 7. Conclusions

This chapter has reviewed the most common strawberry pests in the United States at the time this chapter was written. Pest complexes in other regions of the world will differ. It is probably inevitable that other exotic species will slip through our borders and become pests like the spotted wing drosophila and chili thrips have done. Climate change and changes in agricultural practices, such as the use of high and low tunnel systems, may change the pest complex in different areas of the United States. It is important to be aware of these things when developing an IPM program.

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# Dietary Antioxidants in Experimental Models of Liver Diseases

*Sila Appak-Baskoy, Mustafa Cengiz, Ozgun Teksoy and Adnan Ayhanci*

## Abstract

Oxidative stress is caused by the imbalance between the amount of reactive oxygen species (ROS) and antioxidant capacity in the body. A balanced diet involving the daily intake of antioxidant-rich foods makes improvements in the total antioxidant capacity of individuals and would therefore reduce the incidence of oxidation-related diseases. It may also regulate the degree of oxidative stress. In fact, dietary micronutrients are either direct antioxidants or components of antioxidant enzymes, which may contribute positively to certain indicators of hepatic function. Liver plays an important role in the regulation of various processes such as metabolism, secretion, storage, and the clearance of endogenous and exogenous substances. Once liver is damaged by pursuing a wrong diet and inflammation takes place, most of these physiological functions get altered. Apart from drugs that used to treat the ailments, it is also necessary to determine the pharmaceutical alternatives for the drugs that are used in the treatment of liver diseases. Therefore, this chapter aims to summarize all known information on the effects of dietary nutrients on oxidative stress in experimental liver models.

**Keywords:** dietary antioxidant, free radicals, oxidative stress, liver

## 1. Introduction

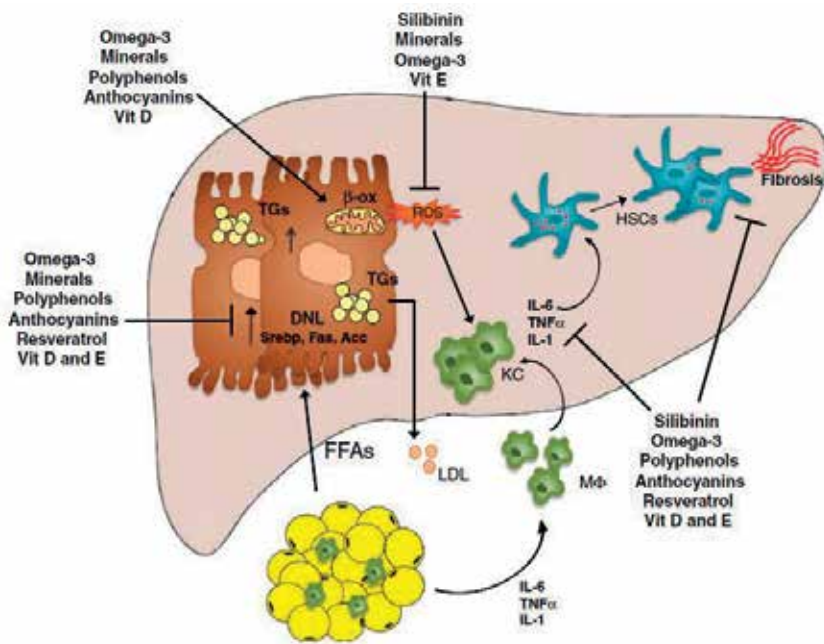
The liver is the major organ metabolizing xenobiotics and endogenous molecules in order to maintain metabolic homeostasis in the organism, which is why it is a target of many toxic substances that cause dysregulated hepatic homeostasis. One of the mostly found clinical liver diseases is nonalcoholic fatty liver disease (NAFLD) (**Figure 1**) [2, 3]. In NAFLD, hepatocytes get filled up with triglycerides, liver expands and its normal functions may get altered. Although it is a disease, the triglyceride accumulation can still be reversed and normal functions may be restored by proper nutrition and exercise. If these lifestyle changes are not pursued, the damage goes on and results in inflammation followed with fibrosis which is unfortunately irreversible.

The liver is made up of hepatocytes, Kupffer cells, liver sinusoidal endothelial cells, pit cells, and hepatic stellate cells (HSC) [4]. Activation of stellate cells by injury caused by many etiological factors would lead to cirrhosis, and it would mark the end stage of progressive fibrosis [5]. Oxidative stress has a vital part in establishing

fibrosis and consequently cirrhosis [6]. For this reason, using molecules with antioxidant properties has been proposed as a treatment for not only fibrosis but also oxidative stress-related cirrhosis. Liver diseases are considered a major medical problem worldwide. There are known to be a large number of liver diseases caused by different insults. Furthermore, the disease type depends on lifestyle factors. For example, the main causes of liver diseases are reported to be viral and parasitic infections in regions like Africa and Asia. For Europe and America, alcohol consumption is thought to be the most important cause of this disease. However, viral hepatitis has showed an increase in recent times in most of the countries [7]. Lifestyle and unhealthy diet is the leading cause of liver diseases in almost all western countries. Until today, no medication is approved for the treatment of this disease; however, improving diet habits and physical exercise works if the disease is not accompanied by inflammation. On the other hand, biologically active food compounds that regulate gene expressions in lipogenesis, fibrosis, and inflammation serve as good therapeutic means to ameliorate these pathological states observed in liver [1] (**Figure 1**).

### 1.1 Oxidative stress

Oxidative stress is recognized as a disproportion between the production of free radicals (FR) and the antioxidant defenses [8]. Increased levels of prooxidants result in damage to the cell in terms of lipid peroxidation as well as oxidative DNA damage



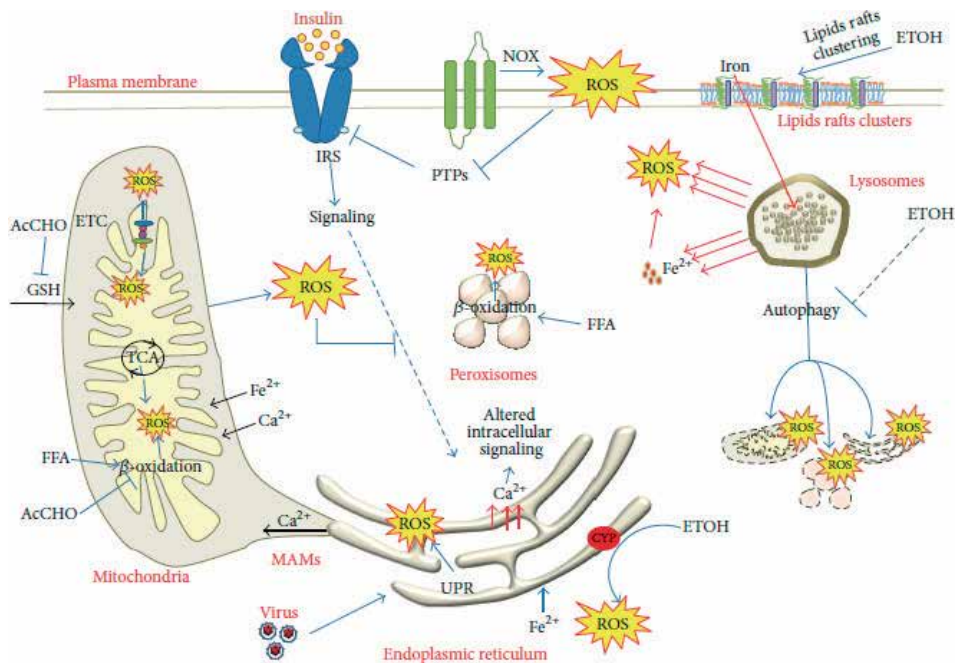
**Figure 1.**

*Molecular mechanisms explaining the hepatoprotective effect of food bioactives. Development of NAFLD/NASH is induced by different risk factors, such as Western-type diet, physical inactivity, and genetic predisposition. In the presence of obesity and IR, there is an increased flux of FFAs to the liver. These FFAs are stored as TG in lipid droplets leading to hepatic fat accumulation or undergo  $\beta$ -oxidation increasing oxidative stress and the inflammatory pathway. The damaged hepatocyte leads to a further increase of inflammatory signaling (IL-1, TNF $\alpha$ , IL-6) and the recruitment of circulating and residual macrophages (KCs). All of these mechanisms can directly induce the activation of HSCs, the major cell type involved in extracellular matrix deposition and liver fibrosis. The bioactive compounds may exert beneficial effects on NAFLD development and progression by inhibiting lipogenesis,  $\beta$ -oxidation of FFAs, inflammation, and HSCs activation. In the cartoon, we have listed the food bioactives indicating the putative mechanisms by which they may improve liver damage in NAFLD [1].*

and thus protein damage [9]. One or more unelectrified FR atoms or molecules may be present as radical cations or radical anions. They are usually unstable and highly reactive because they can react with molecules and abstract electrons. Oxygen can reduce and produce reactive oxygen species (ROS) with exciting electrons, secondary to the interaction of transition metals or by the addition of energy [9, 10]. Oxidative stress causes fibrogenesis by increasing transforming cytokines including transforming grown factor-beta-1 (TGF $\beta$ 1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF $\alpha$ ) [3]. Disruption of the liver metabolism arises from increased quantity of ROS to amplified electron transfer in mitochondrial B-oxidation and increased expression and activity of Cyp2e1 that is from CYP450 family [11]. Intense production of Cyp2e1 is present because of much more consumption of ethanol which is produced by virtue of a lot of direct and indirect mechanisms [12–14] (Figure 2).

## 1.2 Basic sources of free radicals

Cells produce FR as a result of metabolic events; however, this is not the only source that can cause oxidative stress in body. The pollutants in the environment such as toxic chemicals as well as radiation cause a significant increase in amount of FR, ROS, and reactive nitrogen species (RNS) [10]. In the body, variety of different cell types and chemical reactions produce ROS, the most important metabolism is the cytochrome P450 metabolism and mitochondria-catalyzed electron transport reactions. Most of the inflammatory conditions are also responsible from ROS production, and important cell types in these processes are neutrophils, eosinophils,



**Figure 2.** Mechanisms of enhanced ROS production during hepatocyte damage. Ethanol metabolism promotes strong ROS production in the ER by the inducible CYP. It impairs GSH import in the mitochondria, preventing ROS removal. It also impairs B-oxidation promoting lipid accumulation. ETOH induces lipid-raft clustering and increases iron uptake, promoting Fe<sup>2+</sup> leakage from lysosomes and increased Fe<sup>2+</sup> loads in mitochondria and ER, resulting in ROS production. Ethanol also reduced the autophagic removal of damaged cellular components. Viral infection challenges the ER protein folding process leading to ROS production and Ca<sup>2+</sup> leakage in the cytosol and mitochondria. Increased MAMs formation promotes Ca<sup>2+</sup> efflux from ER into mitochondria, increasing mitochondrial ROS production [15].

and macrophages [16, 17]. The chief molecule responsible for the reduction of oxygen in mitochondria is ubiquinone. Mitochondria is such an important organelle to produce ROS and hydrogen peroxide ( $H_2O_2$ ) as it produces 2–3 nmol of superoxide/min per mg of protein, [17]. Different tissues of mammals and different species of mammals have an enzyme called xanthine oxidase, an enzyme belonging to molybdenum, iron-sulfur, flavin hydroxylases that play an important role in the hydroxylation of purines by the oxidation of hypoxanthine to xanthine. Resultant xanthine then oxidized to uric acid. Oxygen reduction takes place in both of these reactions and the first one produces  $O_2^-$ , while the second one produces  $H_2O_2$  [16]. Inflammation serves as another source of ROS generation. During inflammation, activated macrophages increase their oxygen uptake, and this process results in production of  $O_2^-$ , nitric oxide (NO), and  $H_2O_2$  [18]. Another mechanism of  $O_2^-$  production during inflammation is by neutrophils; the enzyme nicotine adenine dinucleotide phosphate [NAD(P)H] oxidase generates  $O_2^-$  that is used to destroy bacteria and this nonphagocytic NAD(P)H oxidases produce  $O_2^-$  in a range of 1–10% [19]. Cytochrome P450 (CYP) enzymes are also important in the production of ROS by the breakdown and/or uncoupling of the P450 catalytic cycle. Hyperoxia would trigger 80% of the  $H_2O_2$  synthesis by microsomes, and under normoxic conditions, peroxisomes produce  $H_2O_2$  but not  $O_2^-$ , and most of the peroxisomal  $H_2O_2$  production takes place in liver [16]. Arginine is reduced to citrulline in a five-electron oxidative reaction by nitric oxide synthases (NOSSs) and this reaction gives rise to NO. Immune cells can also produce NO in the oxidative burst during inflammation. NO can react with oxygen and water in an extracellular environment in order to form nitrate and nitrite anions. Also, the NO and  $O_2^-$  can react together and cause a more reactive FR called peroxynitrite anion ( $ONOO^-$ ) which can cause lipid peroxidation and fragmentation of DNA [20].

### 1.3 Antioxidants

Antioxidants are molecules that can help prevent or delay oxidation of an oxidizable substrate when in low concentrations and they have a high affinity to FR [21]. Antioxidants play an important role to maintain health of the organism by scavenging FR by donating electrons to it. This reduces the reactivity of FR and helps maintain prooxidant/antioxidant balance in cell. A lot of different molecules that have antioxidant activity have been identified. Different natural compounds have so far been studied extensively especially in liver diseases (Table 1).

### 1.4 Curcumin

Curcumin, diferuloylmethane or 1,7-bis (4-hydroxy-3-methoxyphenyl)1,6-heptadieno-3,5-dione is obtained from the rhizomes of *Curcuma longa* (turmeric). Curcumin has many pharmacological properties as it is a strong antioxidant, antifibrogenic, anti-inflammatory, antimicrobial, and anticarcinogenic agent and it also aids in a wound healing [22]. The Food and Drug Administration (FDA) has classified turmeric as a safe substance and toxicity assays done on animals have shown curcumin to be safe even when used in high doses. On the other hand, prolonged high-dose intake of turmeric has been associated with incidences of hepatotoxicity in mice and rats [3]. Curcumin is known to have low bioavailability when administered orally. Arcaro et al. [23] used piperine (inhibitor of hepatic and intestinal absorption) together with curcumin. Even in the presence of piperine, antidiabetic and antioxidant activity of curcumin was not altered. But when higher dose of piperine (40 mg/kg) was used, the beneficial effects of curcumin vanished. On the other hand, Sehgal et al. [24] showed the effect of piperine on curcumin in benzo(a)pyrene toxicity in the liver. They found that pretreatment with 100 mg/kg of curcumin protects against

<b>Antioxidant</b>	<b>Main clinical effects</b>	<b>Clinical relevance</b>
Curcumin	Antioxidant	No studies available in human hepatic disorders
	Antifibrotic	
	Anti-inflammatory	
	Antimicrobial	
	Wound healing	
	Anticarcinogenic	
Resveratrol	Antioxidant	Current data are conflicting, so more clinical studies are needed
	Anti-inflammatory	
	Anticarcinogenic	
	Lipid modulation	
	Antifibrotic	
Coffee	Antinecrotic	Inverse relationship between coffee-cirrhosis has been demonstrated, but more basic research and prospective clinical trials are necessary
	Antifibrotic	
	Antioxidant	
	Anticholestatic	
	Chemoprotective	
Quercetin	Chelation of transition metal ions	No studies available in human hepatic disorders
	Anticarcinogenic	
	Cardioprotective	
	Bacteriostatic	
	Antioxidant	
	Antifibrotic	
	Anti-inflammatory	
	Antiapoptotic	
	Antiaggregatory	
Vasodilating		
Silymarin	Antioxidant	Silymarin has been shown to be effective, but it is necessary to do more clinical trials focused on survival rates of patients with cirrhosis
	Antifibrotic	
	Anti-inflammatory	
	Anticarcinogenic	
	Immunomodulation	
Naringenin	Antioxidant	No studies available in human hepatic disorders
	Hypocholesterolemic	
	Anti-estrogenic	
	Hypolipidemic	
	Antihypertensive	
	Anti-inflammatory	
	Antifibrotic	
	Anticarcinogenic	
	Antiatherogenic	

Antioxidant	Main clinical effects	Clinical relevance
Green tea	Anti-inflammatory	More clinical studies are needed
	Antiarthritic	
	Antimicrobial	
	Antioxidant	
	Neuroprotective	
	Antidiabetic	
	Antiangiogenesis	
L-carnitine	Chelation of transition metal ions	More clinical studies are needed
	Antioxidant	
	Cardioprotective	
	Neuroprotective	
	Anti-inflammatory	
Lycopene	Antioxidant	More clinical studies are needed
	Anti-inflammatory	
	Chemoprotective	
	Anticarcinogenic	

**Table 1.**  
*Nutritional antioxidants.*

a single dose of benzo(a)pyrene; and at this dose, coadministration of piperine had a much better effect than did curcumin alone showing enhancer activity of piperine. In acute and chronic liver injury, curcumin has been shown to have hepatoprotective effects [25]. In 2007, Reyes-Gordillo et al. [26] showed that curcumin is able to inhibit the release of TNF- $\alpha$ , IL-1B, and IL-6. Additionally, curcumin reduces carbon tetrachloride (CCl<sub>4</sub>)-mediated oxidative stress inactivating the nuclear factor-kB (NF-kB) pathway. Moreover, curcumin's hepatoprotective effect takes place by its interactions with Fe<sup>3+</sup> and Cu<sup>2+</sup>. A study by Jiao et al. [27] suggested that curcumin could serve as an iron chelator since transferrin receptor 1 and iron regulatory proteins, indicators of iron depletion, showed an increase with curcumin administration. Charoensuk et al. [28] have indicated that curcumin increases antioxidant capacity of cells by increasing mRNA and protein levels of factors and enzymes such as nuclear factor erythroid 2-related factor 2 (Nrf2), heme oxygenase (HO-1), NAD(P)H quinone oxidoreductase 1 (NQO1), glutamate cysteine ligase (GCL), transcription factor-3, peroxiredoxin 3 (Prdx3), and Prdx6. Curcumin also increases the activity of glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) activity [29, 30]. Curcumin also interacts with enzymes or genes that are important in liver cirrhosis. Hassan et al. [31] showed that curcumin modulates miRNA 199 and 200 which are associated with liver fibrosis in CCl<sub>4</sub>-induced experimental fibrosis model and that curcumin reduced these miRNAs levels close to their basal levels. Finally, in alcohol-induced liver damage, curcumin inhibits the activity of cytochrome P450 2E1 (Cyp2e1) and also its protein levels [32].

### 1.5 Resveratrol

The phytoalexin resveratrol (3,5,4'-trans-trihydroxystilbene) is a polyphenol mostly found in red grapes, red wine, peanuts, and berries [33]. Resveratrol has effects on lipid metabolism, and it also has antioxidant, anti-inflammatory,

anticarcinogenic, and antifibrogenic properties [34]. The rate of absorption of resveratrol is about 75% following an oral administration [35]. Resveratrol is metabolized to resveratrol sulfate, and in its low concentrations, it is converted into resveratrol glucuronide [36] by enzymes glucuronosyltransferase (UGT) or sulfotransferase (ST) [37]. In 2007, Chávez et al. [34] showed that under CCL<sub>4</sub>, resveratrol decreased cytokine TGF- $\beta$  levels and prevented hepatic fibrosis. It also inhibited NF- $\kappa$ B translocation to the nucleus. Resveratrol, as an antioxidant, has protective effects against ethanol-induced lipid peroxidation, toxicity by acetaminophen (APAP), and oxidative stress in animal models of cholestasis [38]. Important player in resveratrol's antioxidant activity is suggested to be run by the OH groups [7]. Blocking OH group methylation showed that resveratrol and trimethylated resveratrol provide some degree of protection, but the latter one has a better protective effect [39]. Another hepatoprotection mechanism of resveratrol comes from its ability to activate genes related to antioxidant system or from its ability to inhibit enzymes. A study by Cheng et al. [40] suggested that resveratrol could activate extracellular signal-regulated kinase (ERK) signaling pathway, which may, in turn, enhance the activation and translocation of Nrf2 to the nucleus, thus increasing the expression of HO-1 and glyoxalase. Price et al. [41] found that resveratrol activates AMP-activated protein kinase (AMPK) and increased nicotinamide adenine dinucleotide (NAD) levels in mice. Zhu et al. [42] have also shown that, in mice, administration of resveratrol increased the antioxidant system (SOD, GPx, and GSH) and also the levels of SIRT1 and p-AMPK were upregulated in liver. Resveratrol has also been shown to inhibit the activity of Cyp2e1 in microsomes of rat liver [43]. Resveratrol also inhibited the activity of P450 isoform APAP-induced liver injury model [44] and activity of Cyp2e1 was also inhibited in diethylnitrosamine (DEN)-induced hepatocarcinogenesis model [45]. The only clinical study that was performed to determine the resveratrol hepatoprotective effect demonstrated that a 500 mg resveratrol dose administered for 12 weeks caused a significant reduction in inflammatory cytokines, serum cytokeratin-18, NF- $\kappa$ B activation, liver alanine aminotransferase (ALT), and hepatic steatosis when compared to the placebo group in patients with nonalcoholic fatty liver disease (NAFLD) [46].

## 1.6 Coffee

Coffee is a mixture of several different molecules such as carbohydrates, vitamins, lipids, nitrogenous molecules, alkaloids, and phenolic compounds [47]. Caffeine, diterpene alcohols (cafestol and kahweol), and chlorogenic acid are the three major compounds found in coffee [48]. Coffee consumption has been linked to the reduction of several chronic diseases [49], probably due to the pharmacological properties that have antinecrotic, antifibrotic, anticholestatic, chemoprotective, and antioxidant functions [50]. Caffeine is the best-known active component of coffee, which is absorbed very rapidly once it has been taken orally (5 min), reaching its peak blood levels after 30 min. When consumed in high amounts, it may produce some side effects. Recommendations from Health Canada in 2013 demanded that the daily caffeine intake for children should not exceed 2.5 mg/kg of the body weight. What is more, tachycardia and arrhythmia typically arise when more than 200 mg of caffeine are ingested [51]. Smith et al. [52] reported in 2002 that the intake of 300 mg of caffeine resulted in a rise in anxiety and tension. Caffeine gets metabolized in the liver. The principal metabolite of caffeine is paraxanthine [53]. An important property of caffeine is that it can easily pass through the blood-brain barrier [54]. Coffee-cirrhosis relationship was shown by Klatsky et al. for the first time [55]. The study showed that the odds ratio for liver cirrhosis tend to decrease from 1.0 for people abstaining from coffee to 0.47, 0.23, 0.21, and 0.16 for 1, 2, 3, or 4 cups of coffee daily, respectively. Although coffee is generally beneficial to the liver, this study failed to show a causative

role of coffee in prevention of liver injury. Therefore, additional basic research and controlled prospective studies are needed in order to show exact effect of coffee on liver tissue. Arauz et al. [50] demonstrated that coffee has a protective effect upon liver injury caused by chronic administration of thioacetamide (TAA). Coffee ameliorated cholestasis and necrosis and this was seen by the measurement of  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GTP), alkaline phosphatase, and ALT levels. Arauz et al. [50] demonstrated in murine models that coffee prevents experimental liver cirrhosis. In these studies, coffee reduced the expression of the profibrogenic cytokine TGF- $\beta$ . Cavin et al. [56] reported coffee to be an inducer of GST, aldo-keto reductase, GSH, HO-1, GST-P1, which are enzymes involved in the detoxification process. Also, they suggested that a possible mechanism of chemoprotection of coffee by stimulating the Nrf2 pathway. In another study, coffee was able to elevate mRNA levels of NQO1 and glutathione-S transferase A1 in the liver and the small intestine [57].

### 1.7 Quercetin

Quercetin, 3,3,4,5,7-penta-hydroxyflavone, is a flavonol especially found in apples and onions [58]. Quercetin chelates heavy metals and has anticarcinogenic, cardioprotective, bacteriostatic, anti-inflammatory, and antioxidant properties [59]; it also functions as a hepatoprotective agent [60]. The normal daily intake of quercetin is less than 5–40 mg. However, if the peels of the foods that contain high amount of quercetin are also consumed, daily intake of quercetin increases to 200–500 mg [59]. In 2004, high-purity quercetin used in foods was grass that serves 10–125 mg of quercetin [59]. The functional groups responsible for quercetin's antioxidant activity were described by Bors et al. [61] in 1990, and they found that orthodihydroxy or catechol groups in the B-ring, a 2,3-double bond of the C-ring, and OH substitution on positions 3 and 5 of the C-ring and A-ring, respectively, are important players in antioxidant action of quercetin [61]. It can interact with both FR and metal ions like  $\text{Fe}^{3+}$  and  $\text{Cu}^{2+}$  for chelation. In a study by Mira et al. [62], it was reported that reduction of  $\text{Fe}^{3+}$  and  $\text{Cu}^{2+}$  takes place by quercetin's 2,3-double bond and the presence of catechol group in the B-ring. Following ingestion, quercetin is rapidly absorbed and its levels in blood peak at approximately in 30 min [63] before it is metabolized by glucuronidation and sulfation by the UGT and ST, respectively.

In experimental fibrosis model in rats, quercetin showed hepatoprotective properties under  $\text{CCl}_4$  treatment that lasted 8 weeks. The hepatoprotective effect of quercetin was found to be mediated by its ability to suppress the expression of profibrogenic expressions of TGF- $\beta$ , CTGF, and collagen- $\alpha$  (Col-1 $\alpha$ ). On the other hand, quercetin also activated enzymes such as metalloproteinases 2 and 9 (MMP2 and MMP9); it also improved the activity of SOD and CAT [64]. Pavanato et al. [65] extended  $\text{CCl}_4$  treatment for 16 weeks and also observed that quercetin improved the hepatic liver enzymes AST, ALT, and inducible NOS (iNOS) expressions; it also decreased collagen amount and reduced lipid peroxidation in liver. Granado-Serrano et al. [66] showed that quercetin modulated Nrf2 and p38 in HepG2 cells. Quercetin has also been shown to suppress the activity of Cyp2e1 in hepatocytes in the presence of ethanol [67]. In line with this finding, in a nonalcoholic steatohepatitis (NASH) model, quercetin was able to reduce Cyp2e1 activity [68].

### 1.8 Silymarin

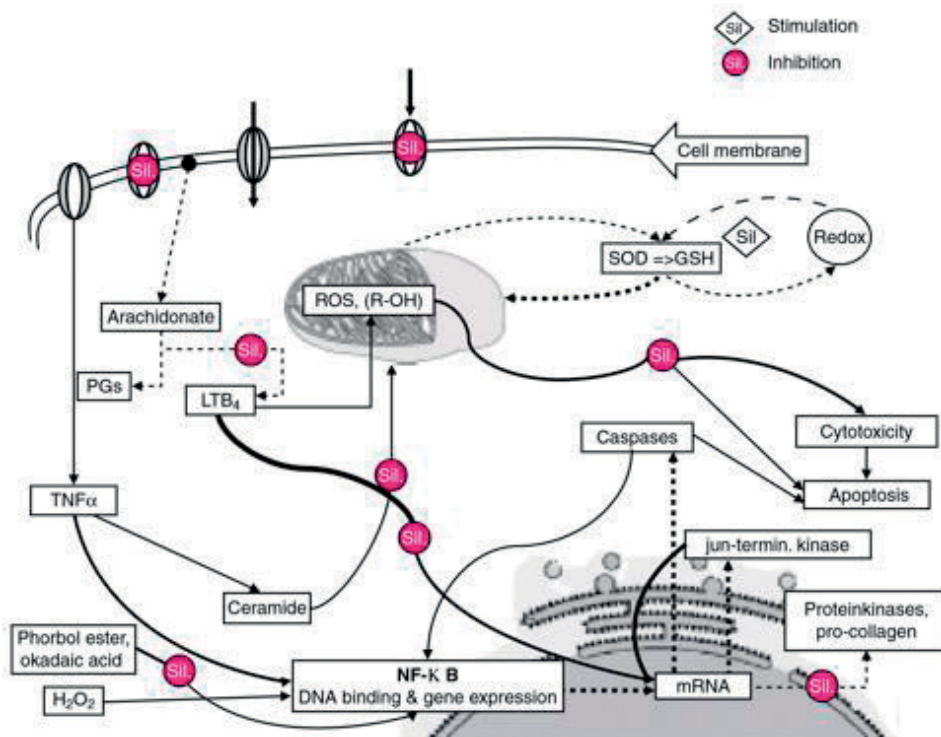
Silymarin, milk thistle or Saint Mary's thistle, is a natural substance obtained from *Silybum marianum* [7]. Silymarin has not been associated with any side effects at acute consumption and the dose range used in literature ranges between 280 and 800 mg/kg of body weight per day. After oral administration, the silymarin peak



plasma concentration is achieved at approximately 6–8 h. The metabolites of silymarin get conjugated in the liver by UGT and ST (phase II reactions) [69]. This substance has many hepatoprotective effects (**Figure 3**). In fact, silybin, a major constituent of silymarin, has found to have iron chelating properties [71]. In a study performed by Najafzadeh et al. [72], hepatoprotective effect of silymarin in iron-overload-induced hepatotoxicity was attributed to its iron-chelator activity; however, no studies have proved the chelating properties per se of silymarin in liver diseases. Silymarin acts as hepatoprotector against several hepatotoxins including D-galactosamine [73]. Silymarin's ameliorating effects on oxidative stress, fibrosis, cirrhosis, and lipid peroxidation are modulated by its phosphatidylethanolamine amount [74]. This hepatoprotective effect is seen with the improvement of liver enzyme activities and levels of cholesterol/phospholipids and also sphingomyelin/phosphatidylcholine ratios in the membrane [75, 76]. Kim et al. [77] showed that silymarin increases nuclear translocation of Nrf2 in activated HSC. Also, silymarin increases the activity of antioxidant enzymes such as SOD, GPX [78], and CAT [79]. A clinical trial examining silymarin in a complex with phosphatidylcholine found reduced levels of the liver enzymes, ALT and  $\gamma$ -GGT, and serum bilirubin levels in a dose-dependent manner in patients suffering from hepatitis due to virus infection or alcohol abuse [80] (**Figure 3**).

### 1.9 Naringenin

Naringenin is also recognized as 5,7,4'-thihydroxyflavanone, and it is a flavanone found in citrus fruits and tomatoes [81]. In a recent study, Yang et al. [82] have



**Figure 3.** A schematic notation of the main pharmacological effects of silymarin in accordance with its hepatoprotective features: The effects of Sm upon cell membranes (upper left) and intracellular cascades are shown here. The metabolic paths are indicated by interrupted lines, while its signal effects are shown in full lines. LTB<sub>4</sub>, leukotriene B<sub>4</sub>; GSH, glutathione; NF-κB, nuclear factor kappa B; PG's, prostaglandins; Sm, silymarin; SOD, superoxide dismutase; ROS, reactive oxygen types; TNF $\alpha$ , tumor necrosis factor  $\alpha$  [70].

reported that naringenin did not cause any harmful effects in beagle dogs, the maximum time of exposure being 180 days and with doses varying of 20, 100, or 500 mg/kg body weight per day. Naringenin has many pharmacological properties. It acts as a hypolipidemic, antihypertensive, anti-inflammatory, antioxidant, and antifibrotic agent [81]. The metabolism of naringenin takes place in small intestine where glycoside form of naringenin gets cleaved, resulting in sulfate and glucuronide metabolites in the small intestine wall; then, it gets absorbed [77]. Mira et al. [62] showed that naringenin can reduce  $Fe^{3+}$  and  $Cu^{2+}$  ions but it is less potent than quercetin. Chtourou et al. [83] found that naringenin averts depletion of SOD, CAT, GPx, and GSH. On the other hand, naringenin also prevents an increase in lipid peroxidation, and it also prevents increase of enzymes ALT and AST [78]. Yen et al. [84] also obtained similar results on liver enzymes and prevention of lipid peroxidation when they used naringenin alone and also naringenin-loaded nanoparticle system (NARN). In both treatments, naringenin exhibited antioxidant and hepatoprotective activities. In these experiments, treatment with naringenin also inhibited the activation of caspases 3 and 8. However, NARN was found to have better hepatoprotective and antioxidant effects than free naringenin, and it was also shown to inhibit caspase 9 during  $CCl_4$ -induced hepatotoxicity in rats. Han et al. [64] reported that a pre-treatment with naringenin-7-O-glucoside increases NQO1 and ERK phosphorylation and translocation of Nrf2 to the nucleus in H9c2 cardiomyocytes. It also upregulated the mRNA expression of GCLC and GCL modifier [64]. Similar findings have been reported by Esmaili et al. [85] who showed that naringenin attenuates  $CCl_4$ -induced liver injury by downregulating TNF- $\alpha$ , INOS, and cyclooxygenase-2 and also by increasing Nrf2 and HO-1 expressions. Motawi et al. [86] showed that naringenin inhibits Cyp2e1 in liver microsomal assay done on rats [86].

### 1.10 Green tea

*Camellia sinensis* or green tea is a widely consumed beverage across the globe and it has antioxidant, anti-inflammatory, antiarthritic, and antiangiogenic effects. It is a mixture of polyphenols (the major class of active compounds) including catechins (also known as flavan-3-ols) which constitute about 30% (mass fraction) of green tea leaves. The major catechins in green tea are (+) -catechin, (-) -epicatechin, (-) -epigallocatechin, (-) -epicatechin-3-gallate, (-) -gallocatechin, (-) -gallocatechin gallate, and (-) -epigallocatechin-3-gallate (EGCG). EGCG is the most abundant catechin accounting for 50% of total polyphenols; thus, it is the main biological active compound of green tea [87]. However, polyphenols are not the only compounds that green tea exerts its antioxidant activity with through. The amino acid, L-theanine, in green tea accounts for 1–2% of the leaf dry weight that is synthesized in the roots of green tea and is concentrated in the leaves. Studies have reported that L-theanine protects the cell by maintaining its GSH levels in cancer and neurotoxic diseases [88]. The intake of green tea can be considered safe unless its consumption exceeds 1–2 cups a day. And higher consumption such as in attempts to lose weight resulted in hepatotoxicity [87]. At normal doses, Pérez-Vargas et al. [88] found that the main amino acid of green tea, L-theanine, reduced expression of NF- $\kappa$ B and downregulated IL-1 $\beta$  and IL-6 and the cytokines TGF- $\beta$  and CTGF. Haleboua-De Marzio et al. [89] tested a single high dose of green tea (400 mg), in patients with HCV-induced cirrhosis and found that it is well tolerated by patients and beneficial for treating cirrhosis.

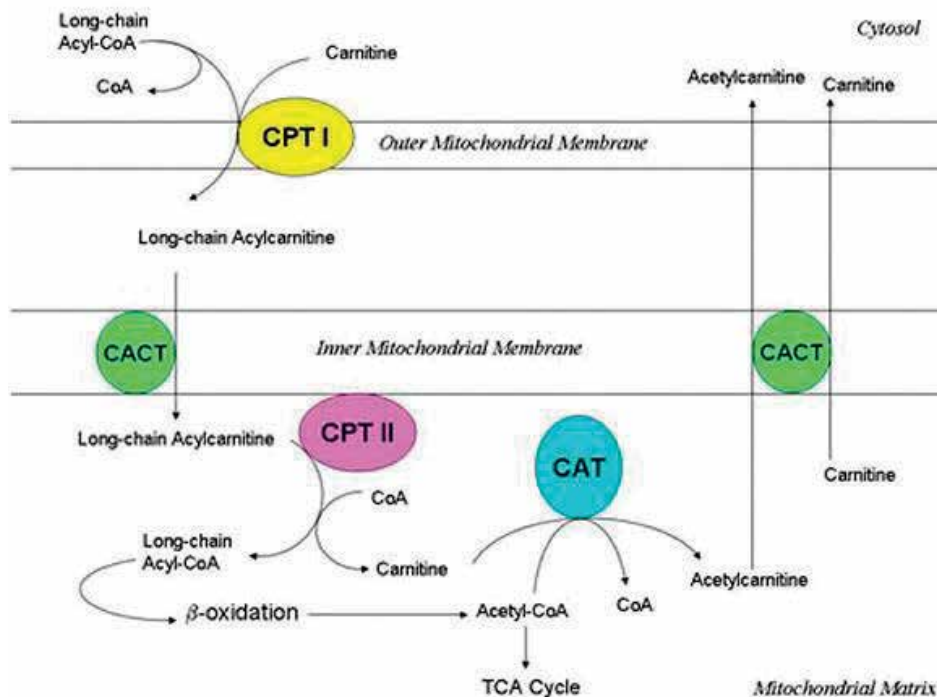
### 1.11 L-Carnitine

L-Carnitine (LC), B-hydroxy-y-trimethylaminobutyric acid, is a water-soluble molecule important in mitochondrial oxidation of fatty acids in mammalian

metabolism (**Figure 4**). LC can exist in three different forms: as free LC, acetyl-L-carnitine (ALC), or other carnitine esters. About 25% of carnitine is obtained from methionine biosynthesis, but most LC is provided by the diet, especially through red meat and milk consumption [91]. LC acts as a carrier of fatty acids across the inner mitochondrial membrane for  $\beta$ -oxidation and ATP production. Apart from its role in the lipid metabolism, LC is also a potent antioxidant, and it protects tissues from oxidative damage. Reduced concentrations of LC in the body are mostly due to the accumulating toxic metabolites and also because of lack of protein in restricted diets. Thus, LC supplementation could be useful not only to supply the tissues in presence of but also in avoiding oxidative damage as a result of increased amounts of reactive species. Since LC can easily cross the blood-brain barrier, LC supplementation may also be beneficial in preventing oxidative injury-related neurological damage and further studies are needed in order to clearly establish LC's role in neurological diseases [92].

### 1.12 Lycopene

Lycopene (LYC) is an acyclic isomer of beta-carotene which has great antioxidant activities. It is synthesized by plants or autotrophic bacteria but not by animals. Red fruits and vegetables, such as tomatoes, watermelons, pink grapefruits, apricots, pink guavas, and papaya, contain LYC. Studies show that LYC consumption not only reduces the risk of cancer of many organs but also retards the growth of tumors. LYC has been shown to have protective effects on other pathologies such as cardiovascular diseases, osteoporosis, male infertility, and this action is mainly mediated by LC's ability to inhibit other toxic agents (**Figure 5**). Numerous *in vitro*



**Figure 4.** The mitochondrial carnitine system. Abbreviations: CPT I, carnitine-palmitoyl transferase I; CACT, carnitine acyl carnitine transferase; CPT II, carnitine-palmitoyl transferase II; CAT, carnitine acyl transferase; CoA, coenzyme A [90].

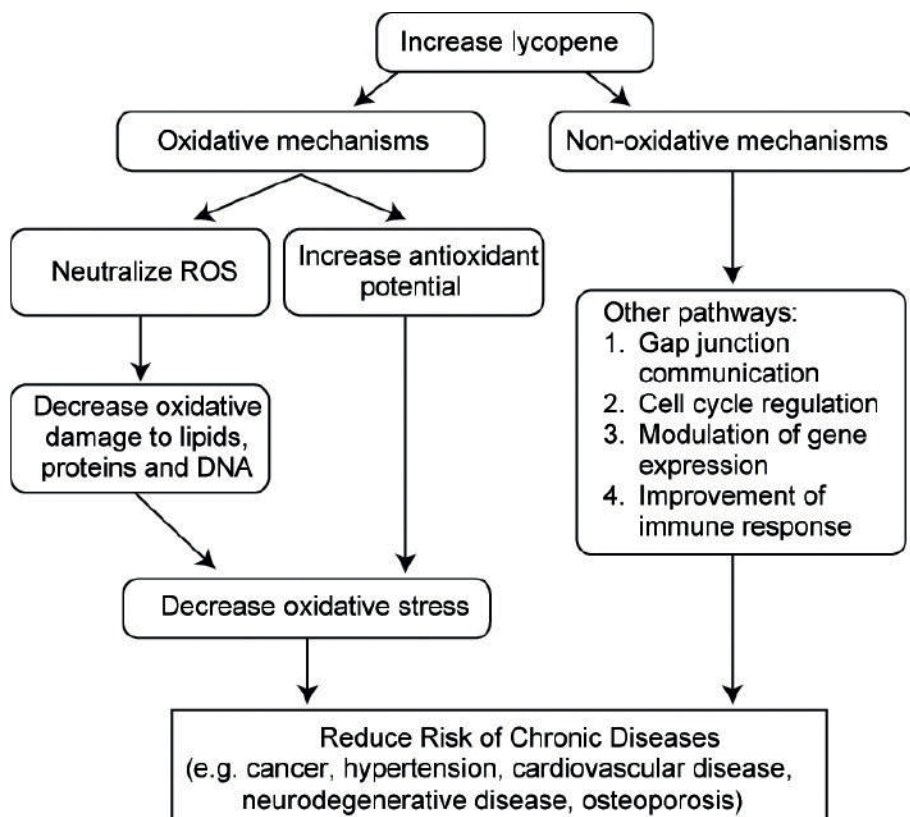
and *in vivo* studies showed that LYC could provide protection against ionizing radiation. Therefore, supplementation of LYC might be protective against damaging effects of radiotherapy in cancer treatments and it can also be protective against accidental radiation exposure [94].

### 1.13 Piperine

Piperine [1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4, pentadienyl] piperidine] is the major pungent alkaloid present in the fruits of *Piper nigrum* L. [95]. Piperine at low concentrations acts as a hydroxyl radical scavenger, but at higher concentrations, it activates the Fenton reaction, resulting in increased generation of hydroxyl radicals. Piperine has hepatoprotective effects and it was shown to inhibit lipid peroxidation in the rat liver microsomes at a concentration of 600  $\mu\text{M}$  [96].

### 1.14 Capsaicin

Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is the major strong and irritating ingredient of red pepper. It may inhibit copper ion-induced lipid peroxidation of human LDL, which suggests that it is an effective antioxidant offering protection against oxidation of human LDL [97].



**Figure 5.** General mechanisms of action of lycopene. The proposed mechanisms of action of lycopene (oxidative and nonoxidative) that decreases the risk of oxidative stress-mediated diseases. Lycopene most likely acts via the oxidative mechanism of action to prevent oxidative stress and its detrimental effects on male infertility. ROS: reactive oxygen species [93].

### **1.15 Garlic and onion**

Diallyl sulfides and diallyl disulfides, the active components of garlic, have anti-inflammatory and antimutagenic activities. Onion is a major source of flavonoids, especially the two quercetin glycosides, quercetin 4-*o*- $\beta$ -glucoside and quercetin 3,4-*o*- $\beta$ -diglucosides, which are recognized as bioactive substances. In order to show the antioxidant properties and protective effects of garlic and onion, a study was carried out on rats. Animals were treated with 0.6 mg nicotine/kg and also given 100 mg garlic or onion oils/kg for 21 days. Nicotine increased concentrations of thiobarbituric acid, conjugated dienes, and hydroperoxides in the tissues. Supplementation with both the garlic oil and onion oil increased resistance not only to lipid peroxidation but they also increased levels of antioxidant enzymes and glutathione. These conclusions state that oils of garlic and onion are effective antioxidants against nicotine-related oxidative stress and damage [98].

### **1.16 Vitamins C and E**

Vitamin C, substrate for ascorbate peroxidase, is not only a highly effective antioxidant but also an essential component of a healthy diet. Vitamin E, the major antioxidant found in lipid composition of membranes, is a fat-soluble antioxidant. During fat oxidization, vitamin E helps to inhibit formation of ROS [99]. Several studies showed that vitamin E serum levels are significantly reduced in alcoholic liver disease [100]. It is also shown that vitamin E levels are inversely proportional to formation of oxidative stress products that correlate with the extent of liver damage [101]. For this reason, maintenance of normal concentrations of vitamin E appears to be necessary for preventing lipid peroxidation due to alcohol consumption. Works from several laboratories have so far indicated that mitochondrial damage may present a common early event in cell injury [102]. It is possible to prevent mitochondrial damage through vitamin E [103]. Vitamin E or C alone, or in combination, can ease scavenging free radicals that are generated in the liver tissue [104]. In the mouse model, vitamin E supplementation restores alcohol-induced redox status, reduces apoptosis, and prevents oxidative stress [105]. What is more, vitamin E is effective in doses of 600 mg daily when it comes to suppressing HBV replication and normalizing ALT in a significant proportion of chronically infected patients with CLD [106].

### **1.17 Trace minerals**

Trace minerals act as a cofactor of antioxidant enzymes thus enabling the antioxidant activities to take place. These trace minerals include selenium (Se), zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu) [103].  $O_2^-$  radicals are eliminated by the enzyme Cu-Zn-SOD and Cu and Zn are the co-factors for the enzyme. One of the enzymes responsible for  $H_2O_2$  clearance from the cells is CAT and Fe is the essential cofactor of this enzyme. Levels of ferritin may decline with exercise and increasing dietary or supplemental Fe can improve performance. It was shown that moderate-level supplementation of Fe to competitive swimmers increased their performance and helped to maintain normal ferritin levels [107]. Selenium (Se) is a cofactor for the antioxidant enzyme GPx, which is like the enzyme CAT, responsible for removing  $H_2O_2$  and other organic  $H_2O_2$  from the cell. A study by Akil et al. [108] showed in rats, that upon acute swimming exercise, lipid peroxidation in the brain was increased and Se supplementation to these rats increased antioxidant activity resulting in inhibition of the free radical production [108]. Manganese (Mn) is a cofactor for the enzyme Mn-SOD. It eliminates  $O_2^-$  radicals produced during oxidative phosphorylation [109].

## 2. Conclusion

Investigations done on antioxidants have shown that these compounds are candidates for the treatment and candidates to prevent oxidative stress-related diseases. This chapter focuses on antioxidants that can be investigated in experimental and clinical trials of many diseases but especially in diseases of liver. Main nutritional components involved in the production and/or removal of free radicals and the role of free radicals in the pathogenesis of several hepatic diseases and related comorbidities have been described in this chapter.

Among the antioxidants that were described, curcumin, naringenin, and quercetin have been found to be effective antioxidants in treatment of experimental liver injury. Green tea has been shown to protect against different kinds of cancer in clinical trials but not on hepatocellular carcinoma. Resveratrol has been extensively studied in experimental models of liver diseases and has been shown to have protective effects on fibrosis. So far, there are not much clinical trials on ameliorating and disease preventing effects of most potent antioxidants on liver and these antioxidants are good candidates for clinical trials not only because they show great disease preventing and ameliorating effects but also because they are derived from food sources and have a good metabolic tolerance.

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This book mainly deals with pre- and postharvest management practices of the strawberry to ensure that high-quality fruits are delivered to the consumer. The influence of climatic variables, cultural practices, harvesting techniques, and use of chemicals and other natural compounds on fruit quality are discussed. Factors affecting fruit growth and development and processes regarding maturation and biochemical changes during fruit ripening are also presented in one of the chapters of this book. Some chapters provide information regarding harvesting, storing, packaging, transporting, and also selling that affect strawberry quality greatly. Enhancement of yield and antioxidant contents in the strawberry by various natural products, including chitosan and probiotic bacterial, are also included in this book. The final chapter states that antioxidants present in strawberry fruit play a dietary role in alleviating oxidative stress in experimental liver models. This book focuses on the postharvest quality management of the strawberry and provides a useful resource to educationists, traders, and commercial strawberry growers.

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