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Abstract

Strawberries, raspberries, and blackberries are highly appreciated fruits due to their unique taste and high content in antioxidant and bioactive compounds. They are rich in phenolic compounds, mostly flavonoids and anthocyanins, which are responsible for fruit color and can exert antioxidant, antimicrobial, anti-inflammatory, anticancer, and cardioprotective effects. However, berries have a short storage life, as a result of their high respiration and softening rate, and susceptibility to mechanical damages and decay. As berries are considered non-climacteric fruit, they must be harvested at, or near to full maturity, because they will not continue to ripen normally once detached. At this stage, the fruit presents appropriate organoleptic attributes but may become softer and more sensitive to mechanical damage. Thus, it is crucial to be extremely careful during harvest and postharvest handling and to sort, grade, and pack the berries in the field, avoiding excessive manipulation of the fruit. The most extended methods to maintain quality during the postharvest period are prompt precooling and storage at low temperatures. Modified and controlled atmospheres with up to 20-kPa CO₂ and 5–10-kPa O₂ reduce microbial growth and delay senescence but can affect bioactive compounds with a cultivar-dependent response observed for these technologies.

Keywords: berries, maturity index, packaging, refrigeration, storage

1. Introduction

Berry fruits include, among others, strawberries (Fragaria ananassa), raspberries (Rubus idaeus), and blackberries (Rubus spp.). These fruits are characterized by their acidic taste and can be consumed fresh or frozen. Fresh fruits are mainly consumed locally and are available only in the ripening season, except countries from South America, like Colombia or Ecuador, where the production occurs all year round. Berries are also available as processed products like refrigerated fruit pulp, jams, juices, and nectars [1]. What's more, due to their high content in antioxidant and bioactive compounds, they can be considered as functional foods. In
effect, different studies conducted on berry fruits report antioxidant, antimicrobial, anti-inflammatory, anticancer, and cardioprotective effects, which were attributed to their high content in bioactive compounds, mainly different phenolic compounds [2].

However, the manipulation of these fruits presents a series of challenges: berries lack a protective peel and are highly perishable, mainly because of their susceptibility to mechanical damage, water loss, and fungal decay [3]. What’s more, berries are considered non-climacteric fruit, which implies that they need to be harvested at, or near to, full maturity as most of them will not continue to ripen normally once detached, and eating quality will not improve after harvest. In some cases, they can color in storage but if they are harvested too early, texture, sweetness, and acidity fail to fully develop [4].

Fruit quality for the market is largely determined by physicochemical parameters like size, full color, gloss, firm and crisp texture, absence of decay, injuries and bruises, a balance between sweetness and acidity, green sepals, and typical aroma. At the same time, the main causes of loss and rejects include weight loss, presence of bruises and cuts, symptoms of mold and decay, color changes, juice leakage, and sepal wilt.

To get the maximum quality at harvest and maintain this quality during transport and commercialization until the fruit is consumed, it is essential to harvest berries at the optimum stage of maturity [5]. In this sense, the UNECE Standard FFV-57 [6] concerning the marketing and commercial quality control of berry fruit establishes that “Berry fruits must be sufficiently developed and display satisfactory ripeness according to the species but must not be overripe,” emphasizing the need to harvest at the appropriate maturity stage for each type of fruit.

2. Harvest

In order to avoid excessive manipulation and damage to the fruit, berries for the fresh market should be hand-harvested, sorted, graded, and packed in the field, directly into the final container. Fruit ripeness at harvest and fruit handling are two critical factors in the postharvest keeping quality. In fact, the stage of maturity at harvest largely affects the shelf-life of berries, their storage behavior, and sales probability [7]. Immature fruit may have a longer storage capability but are unlikely to develop appropriate organoleptic characteristics while shelf-life of over-mature fruit is generally very short as the susceptibility to decay also increases [8].

As berries ripen quickly but non-uniformly (Figure 1), it is crucial to harvest frequently (daily, or every 2–3 days, depending on weather conditions and area of production) and also train pickers to identify the proper ripening stage and in the correct harvest practices to avoid damages to the fruit.

Ideally, the fruit should be harvested early in the morning, after the dew is off the berries or in the evening when the temperatures are cooler [9]. Berries should not be touched before harvest because they are extremely fragile and easily damaged during harvest, for example, by finger pressure. Only sound berries with good appearance should be placed in the packages,
and once harvested, fruit must be protected from exposition to direct sunlight. Rotten fruit must be picked off plants and discarded far from the marketable berries to avoid contamination of the latter while small and overripe fruits can be used for processing [10].

2.1. Harvest maturity

Different maturity indexes can be used for determining berries’ optimum harvest date. However, harvest maturity is mainly determined by fruit surface color and most standards require for strawberries that more than one-half to three-fourth of the surface to be colored. In the case of raspberries and blackberries, the fruit must present a completely red and a bright, dark purple/black color, respectively. Color is also the main criterion used by the consumer to judge fruit quality [11]. Besides color, blackberries, and raspberries should pull easily from the receptacle yet being still firm. Regardless of the berry considered and in addition to color, appearance (size, shape, and absence of defects), firmness, flavor (soluble solids, titratable acidity, and flavor volatiles), and nutritional value (vitamin C) are all important quality characteristics that must be considered.

Several studies have shown that the color of these berries can change during storage even if the fruit are harvested at early stages of color development [12–14]. However, the changes in sugar and acid content of these unripe fruits are not enough to make them suitable for fresh consumption [4]. On the other hand, Krüger et al. [7] reported that suitability for selling raspberries declined rapidly with increased ripening stage, and thus, the fruit should not be stored and must be sold and consumed immediately after picking.
In any case, it is recommended to avoid mixing different ripening stages in the same pack, as this practice is usually rejected by consumers at the marketplace. At an industrial level, fruit selection is based both on external attributes such as intense red color and color distribution, fruit size and shape, and absence of physiological defects and on internal quality parameters including sweetness, acidity, and flavor [15]. For an acceptable flavor, a minimum of 7% soluble solids and/or a maximum of 0.8% titratable acidity are highly recommended for strawberries [16]. Similarly, the Ecuadorian Quality Standard NTE-2427 [17] for Andean blackberries (Rubus glaucus Benth) establishes a minimum of 9% soluble solids, a maximum of 1.8% titratable acidity and a minimum of 5 for the maturity index (total soluble solids/titratable acidity).

2.2. Packing

The containers most commonly used at the supermarket for raspberries and blackberries are plastic clamshells containing 250 g of fruit. In the case of strawberries also, containers for 500, 1000, and even 2000 g of fruit are used, depending on fruit size (Figure 2a and b). Pulp and wooden containers are also used, but they present the disadvantage that stain easily, and wooden containers are also expensive. Regardless of the material, wide and shallow containers are preferred to deep containers and no more than three layers of fruit should be included in each package as the fruit in the bottom may be crushed by the fruit on top.

![Plastic clamshells of 250 (a) and 500 g (b) for the packing of raspberries and blackberries, and strawberries, respectively.](image-url)
Plastic clamshells present some advantages: they are solid and thus give protection to the fruit from mechanical damage, they do not stain, they are inexpensive, and, as they are usually clear or transparent, they allow consumers to inspect all the fruit at the time of purchasing. The containers must be vented on top and sides and have a lid to reduce mechanical damage and moisture loss. On the other hand, the main disadvantage of these packages is plastic disposal after use.

In some countries, it is still also a very common practice the use of baskets or buckets, sometimes containing up to 12–15 kg of fruit (Figure 3). Customers pay for the first basket and bring them back in following purchases. This kind of containers are not appropriate as they are usually not washed or disinfected before reuse and thus lack hygiene and can accumulate fungal spores. Furthermore, the excessive weight of fruit causes damage to the fruit located at the bottom which usually collapses. In order to absorb the juice leaked from the fruit, non-food grade, periodic paper is frequently added at the bottom of the baskets, underneath the fruit, worsening the situation. Some efforts are being made to eliminate these kinds of practices and to replace these containers by cartons containing plastic clamshells or cardboard boxes (Figure 4a and b).

Figure 3. Baskets used for blackberries harvest.
3. Precooling

Precooling, consisting in rapid removal of field heat immediately after harvest, is essential to maintain fruit quality and control decay [18]. For example, strawberries rapidly cooled down to 0°C showed threefold the storage life of those fruit maintained at 10°C [19]. Field heat is often removed using forced air cooling, where rapidly moving cold air is forced through pallets of fruit to lower fruit temperature to 0 to 1°C within 2 h of picking. This method is preferred to room cooling, as forced air can cool berries to 1°C within an hour, whereas room cooling may take up to 9 h [9].

High relative humidity (85–95%) should be maintained within the refrigerated rooms, but free moisture on the berries or in the containers must be kept to a minimum as, to reduce fruit rot, the berries must be kept dry. Precooling conditions for blackberries are forced air cooling to 5°C within 4 h and fruit should be transported at refrigeration temperatures of 5°C or less. Raspberries should be forced air cooled to 1°C, no later than 12 h after harvest.

4. Storage

Strawberries, raspberries, and blackberries are highly perishable due to their relatively high water content, high physiological postharvest activity, and susceptibility to fruit rot and darkening. The high respiration rates of these fruits cause changes in texture, color, flavor, and nutritional content during storage, and such changes are crucial for the determination of fruit quality and consumer's acceptability [20]. Their short storage life is also the result of decay caused by rot-causing pathogens and quick softening rates [21]. *Botrytis cinerea*, a necrotrophic fungus that causes gray mold rot, is one of the main pathogens responsible for postharvest decay in berries. The symptoms of disease are soft rot with a collapse and water soaking of parenchyma tissues, followed by the appearance of gray masses of conidia [22, 23] (Figure 5).

The presence of ethylene in storage can stimulate respiration rates and gray mold growth. Moreover, color of raspberries can be adversely affected by ethylene as it causes darkening of the red fruit to purple-red [10].
Another physiological disorder that can affect berries during storage is water loss, which in turn causes fruit shriveling, loss of gloss, and plays an important role in anthocyanin degradation. Water loss accelerates senescence of the fruit and the maximum permissible amount of water that can be lost (based on weight loss) from the fruit before becoming unmarketable is 6%. During postharvest handling of the berries, water loss can be reduced by prompt precooling and adequate packaging and storage at optimum temperature and relative humidity [24].

At present, the most extended methods used to maintain quality and bioactive compounds stability and to control decay of fruits and vegetables are postharvest washing, rapid cooling immediately after harvest, and storage at low temperatures [25, 26]. Furthermore, postharvest diseases are normally controlled by the use of synthetic fungicides [27] and storing under controlled or modified atmospheres with high CO$_2$ concentrations [28]. However, these methods present some limitations. Berries washing before retail is not recommended because the skin of the fruit may be damaged easily and the drying period delays precooling and enhances infections by pathogenic microorganisms [27].

Likewise, chemical fungicides may exert several negative effects on food safety and the environment, and there are public concerns about environmental pollution, possible contamination of berries by fungicide residues, and the inability to control fungal diseases because of the appearance of fungicide-tolerant strains of pathogens [29].

4.1. Temperature

One of the main factors affecting the storage shelf-life and quality of fruit and vegetables is temperature, as it regulates the rate of all the metabolic processes that occur in these products.
Low temperatures slow down fungal growth and, at the same time, reduce respiration rate and water loss and, therefore, delay ripening and senescence processes [30]. As these berries are insensitive to chilling injury, extending the shelf-life of berry fruit is often achieved through low temperature with optimum storage conditions for strawberries, raspberries, and blackberries being 0°C and 90–95% relative humidity [31].

Storage temperature is one of the key factors in suppressing fungal decay and influencing the stability of phenolic antioxidants in fruits during postharvest storage [32]. Also, temperature management is the most important factor to be taken into account to retain the initial ascorbic acid content during storage. Nevertheless, even when temperatures around 0°C are considered the best for berries’ storage, the distribution in trucks and shops, the commercialization, and the storage in consumer households usually occur at higher temperatures, which can affect the berries shelf-life and their physicochemical quality and nutritional value, in terms of soluble sugars, vitamin C, and antioxidant compounds [33, 34].

Despite the already-known positive effects of low temperatures on postharvest shelf-life and quality of fresh fruits and vegetables, contradictory results can be found in the literature. Cooling of fruit at 0°C can be detrimental for short-term sales, as fruit appearance may be duller, and condensation of fruit during re-warming might result in greater decay incidence [35]. Jin et al. [36] indicated that strawberries stored at 10°C had higher antioxidant enzymes activities, higher level of phenolics and anthocyanins contents, and stronger oxygen radical scavenging capacities than those stored at 0 or 5°C, and Kalt et al. [32] found that low temperatures could affect anthocyanin synthesis during storage of small fruits. Similarly, anthocyanin and ascorbic acid biosynthesis was delayed in three strawberry cultivars stored at 6°C in comparison with storage at 16°C, while the contents of flavonols, ellagic acid, and total phenolics were not affected by the temperature lowering [33].

In blackberries, Joo et al. [37] found a reduction in total anthocyanin content (TAC) after 18 days at 3°C, while Wu et al. [38] did not see a clear tendency in the evolution of anthocyanins during 7 days at 2°C. On the contrary, in Andean blackberries harvested at the light and dark-red maturity stages, we observed an increase in total anthocyanin content during 10 days of storage at 8 ± 1°C and similar results were reported by Kim et al. [39] who observed that TAC increased after 15 days of storage at 1 or after 13 days at 1 plus 2 days at 20°C.

On the contrary, Piljac-Žegarac and Šamec [2] reported that the marketable quality of strawberries was preserved at 4°C for a prolonged period of time in comparison with storage at room temperature, while higher antioxidant capacity values were maintained at the lower temperatures, as opposed to 25°C. Similarly, storage of strawberries at 1°C together with moisture loss control reduced losses of total ascorbic acid by 7.5-fold compared to fruit stored at 20°C [40]. These authors concluded that even short periods at ambient temperature without control of water loss could result in considerable losses of total AA in strawberries. Moreover, Shin et al. [35] reported that the best temperature for visual appearance of strawberries was 0.5°C, but for short-term storage periods (up to 4 days), it was also possible to use moderate temperatures of 10°C. This temperature was useful to delay fruit ripening compared to room temperature and, at the same time, provided a balance between sensory attributes and those associated with the nutritional status of the fruit.
In Andean blackberries, we also observed that by storing the fruit in refrigerated storage, weight and firmness loss were reduced and microbial growth was delayed in comparison with storage at room temperature. What’s more, the refrigerated fruit presented higher scores in the sensory analysis and, the total phenolic content and the antioxidant activity of the fruit were not affected by the cold storage.

4.2. Modified and controlled atmospheres

Modified atmosphere (MA) and controlled atmosphere (CA) refer to any atmosphere different from the normal air and usually involve atmospheres with reduced O₂ and/or elevated CO₂ levels. The difference between them is that CA is strictly controlled during all time.

Both MA and CA can be used for the storage, transport, and packaging of different types of food in compliment to low temperatures to extend their shelf-lives after harvest. Exposure of fresh horticultural crops to low O₂ and/or elevated CO₂ atmospheres within the range tolerated by each commodity reduces their respiration and ethylene production rates and therefore results in several beneficial effects such as delay of ripening and senescence and associated biochemical and physiological changes, reduction of sensitivity to ethylene action, alleviation of certain physiological disorders such as chilling injury, direct, and indirect control of pathogens, and consequently decay incidence and severity. On the contrary, if horticultural products are exposed to O₂ concentrations below, and/or CO₂ concentrations above their optimum tolerable range, the initiation and/or aggravation of certain physiological disorders, irregular ripening, increased susceptibility to decay, development of off-flavors, and eventually the loss of the product can occur [41].

Modified atmospheres (MA) and controlled atmospheres (CA) with elevated (15–20%) carbon dioxide and 5–10% oxygen concentrations reduce the growth of *Botrytis cinerea* (gray mold rot) and other decay-causing organisms. In addition, it reduces the respiration and softening rates of berries, thereby extending postharvest life. Nevertheless, further reductions of O₂ concentrations to 2 kPa had no benefit and may cause fermentation of the fruit [42].

In addition, off odors can be produced if the fruits are kept under high CO₂ atmospheres for more than 4 days as a result of anaerobic respiration [43] and the effect on the flavor preservation of these fruit is not clear. Several authors [28, 44, 45] reported changes in pH, titratable acidity, total soluble solids, sugars and organic acids, and fermentative metabolites after storage under CO₂-enriched atmospheres. In effect, different fermentative volatiles (acetaldehyde, ethanol, and ethyl acetate) were found after storage of strawberries in air + 20-kPa CO₂ at 2.8°C [46]. Among aroma compounds, esters are apparently the volatiles most affected by CO₂-enriched atmospheres [47].

Anthocyanin synthesis continues after harvest, but it is inhibited in fruits stored in high CO₂ atmospheres for more than 4 days as a result of anaerobic respiration [43] and the effect on the flavor preservation of these fruit is not clear. Several authors [28, 44, 45] reported changes in pH, titratable acidity, total soluble solids, sugars and organic acids, and fermentative metabolites after storage under CO₂-enriched atmospheres. In effect, different fermentative volatiles (acetaldehyde, ethanol, and ethyl acetate) were found after storage of strawberries in air + 20-kPa CO₂ at 2.8°C [46]. Among aroma compounds, esters are apparently the volatiles most affected by CO₂-enriched atmospheres [47].

Anthocyanin synthesis continues after harvest, but it is inhibited in fruits stored in high CO₂ concentrations. According to Holcroft and Kader [28], high CO₂ concentrations together with low O₂ concentrations can also affect adversely total ascorbic acid and anthocyanin contents and, thus, have a negative impact on fruit color and nutritional value. Conversely, the firmness, the external color, and the total phenolic compounds content of Selva strawberries were not affected by storage atmospheres with up to 20-kPa CO₂ [28]. In both, strawberries and raspberries, a cultivar-dependent response to changes in the storage atmosphere was observed.
An atmosphere of 12.5-kPa CO$_2$ and 7.5-kPa O$_2$ was effective in reducing decay in red raspberries and elevated concentrations of CO$_2$ together with reduced concentrations of O$_2$ were shown to inhibit mycelial growth and spore germination of _B. cinerea_ and other fungi responsible for postharvest decay of fruit [42]. What’s more, raspberries stored in 10/15-kPa O$_2$/CO$_2$ presented a more attractive color in comparison with fruit stored in air.

Finally, Giovanelli et al. [48] reported that the use of high and medium barrier materials delayed senescence and did not affect negatively the nutritional and antioxidant properties of red raspberries stored at 4°C. However, fermentative volatiles were found for these fruits, especially when an oxygen absorber was included in the packages.

5. Alternative methods for decontamination of strawberries

The need to minimize chemicals use has encouraged the rapid development of alternative techniques [29]. One of the new approaches is the use of ‘generally recognized as safe’ (GRAS) products, such as UV radiation and ozone, due to minimal concerns about their environmental impact and low residues in the treated commodity.

5.1. UV-C radiation

One strategy that can be an adjunct to refrigeration is the exposure of fruits to hormetic doses of UV-C radiation, a physical treatment that has been tested in strawberries and other fruits to control postharvest diseases [43, 49, 50] and delay some ripening-associated processes [51, 52]. Hormesis has been defined as the use of potentially harmful agents at low doses in order to induce a beneficial stress response [53].

UV-C seems to have a direct germicide effect on pathogens and an indirect effect by inducing defense mechanisms in the plant tissues [53–55]. Irradiation with UV-C is known to stimulate the phenylpropanoid pathway in several fruits, mainly by the induction of phenylalanine ammonia lyase (PAL), a key enzyme in this pathway [56]. The compounds synthesized by this pathway are implicated in a protective role against pathogens through reinforcement of plant cell walls, direct inhibition of growth, and/or inactivation of enzymes that contribute to tissue maceration [57].

Particularly in strawberry fruit, different UV-C doses increased enzyme activity, the antioxidant capacity and total phenolic content during storage, which correlated with lower fruit decay observed in treated fruit [58]. The synthesis and accumulation of phenolic compounds following irradiation with UV-C could also play an additional indirect role in fruit protection acting as natural substrates of polyphenol oxidase (PPO). One of the proposed roles of the reaction products of PPO (quinones) in plant defense is their action as bactericidals and fungicidals [59]. It was found that postharvest UV-C treatment, a few hours prior to inoculation with _B. cinerea_, reduced the percentage of fruit infection in strawberries during storage [60]. These authors also reported that irradiation of fruit with UV-C increased expression and activity of several enzymes (PAL, peroxidases, PPO, chitinases, and β-1,3-glucanases) which are involved in defense mechanisms against pathogens and abiotic stressors. In another
experiment, photochemical treatment with UV-C delayed the appearance of gray mold rot in stored strawberries by up to 5 days at both 4 and 13°C [43]. These authors observed that the treatment with UV-C also enhanced the accumulation of anthocyanins, which in turn contributed to redder and visually more appealing fruit. In contrast, Erkan et al. [58] found little effect of UV-C treatments on anthocyanin content in strawberries.

5.2. Use of ozone

Another emerging technology with potential application in the food industry is the use of ozone as a sanitizer [61]. O₃ can be used for the postharvest treatment of fresh fruits and vegetables, in air or water, or as a continuous or intermittent atmosphere throughout the storage period. Gaseous ozone can be used to sanitize storage rooms and to prevent bacteria, molds, and yeasts development on the food surfaces. It can also eliminate undesirable flavors produced by bacteria and chemically remove ethylene gas to slow down the ripening process [62].

Ozone gas efficacy to inactivate microorganisms is conditioned by the species considered, its growth stage, the ions present in the air, the O₃ concentration and exposure time, and, the temperature and relative humidity of the room [63]. In air, the reactivity of ozone is greatest with fungi, molds, and some odor-causing chemicals and least with dry spores and bacteria. For optimum efficiency, it is also essential that the gas is thoroughly and evenly distributed quickly. Otherwise, decomposition will occur before the O₃ is able to contact its target [62].

Washing strawberries with ozonated water (0.3 ppm, 2 min) was an effective treatment to reduce microbial counts and enhance anthocyanin and ascorbic acid retention of these fruits during 13 days of refrigerated storage [64]. Similarly, Zhang et al. [65] reported greater ascorbic acid retention in strawberries treated with gaseous O₃ (4 ppm, 30 min/day) in comparison with untreated fruit. Moreover, strawberries' levels of biothiols were not affected by the treatment with either gaseous-phase or aqueous-phase ozone [66]. Finally, while total phenolic and ellagitanin contents were similar in O₃-treated and untreated strawberry fruit after a storage period of 12 days, the procyanidins and anthocyanins contents were reduced by the exposure to this gas [67].

In addition to their antimicrobial power, O₃ and UV-C radiation gather other advantages, which turn them into appealing and environmental friendly technologies [61]. Neither ozone nor UV-C leave undesirable residues on food or food-contact surfaces nor create undesirable disinfection by-products [68]. Moreover, the application of these sanitizers in food processing is approved by the code of Food and Drug Administration (FDA) in the USA and is allowed by organic certification [69].

There are numerous studies in the literature reporting on the use of both, O₃ and UV-C light, on several fruits and vegetables. However, results are sometimes contradictory and information about the effects of these decontamination treatments on sensory and nutritional quality or health-promoting composition of treated products is scarce. It should be taken into account that while high doses of oxidizing agents may result in depletion of natural antioxidants, moderate or low doses of oxidative stress were shown to cause a protective response, enhancing the level of endogenous antioxidants [70, 71].
6. Transport

Berries must be transported in clean and well-maintained trucks, and it is crucial to maintain the fruit cold and wrapped during loading, unloading, and transportation. In order to ensure a proper circulation of the cold air, the flats or boxes must be stacked on pallets and without touching the truck walls. Frequently, rural roads are not in optimum conditions causing bruises and abrasion due to the truck vibration and the impacts between the packed fruit and between the fruit and the walls of the packs. These mechanical damages can be minimized by stabilizing the load on every pallet, for example, by using stretch film and by using trucks with air suspension systems.

When refrigerated transport is used, it must be considered that trucks’ mechanical refrigeration equipment is designed to maintain temperature but they do not have the capacity to lower the temperature of the produce. So, it is very important to achieve the proper cooling of the product before loading. Finally, to avoid condensation on the fruits, berries should be allowed to warm only when they are ready for display to consumers and before removing the plastic wrap over the flats [10].

Author details

Sandra Horvitz

Address all correspondence to: sandra.horvitz@unavarra.es

Food Science and Engineering Faculty, Technical University of Ambato, Ambato, Ecuador

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