Strategies to reduce microbial risk and improve quality of fresh and processed strawberries: A review

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Abbreviations: AIT: allyl isothiocyanate, CAP: cold atmospheric plasma, CFU: colony-forming units, EFSA: European Food Safety Authority, EOW: electrolyzed oxidizing water, HAV: hepatitis A virus; HDL: high density lipoprotein-cholesterol, HPP: high pressure processing, IPL: intense pulsed light, LAE: lauric arginate ester, LDL: low density lipoprotein-cholesterol, LVA: levulinic acid, MAP: modified atmosphere packaging, MNV-1: murine norovirus 1, NoV: norovirus, PAA: peracetic acid, PEF: pulsed electric field, POD: peroxidase, PPO: polyphenoloxidase, RASFF: Rapid Alert System for Food and Feed, SDS: sodium dodecyl sulphate, TAB: total aerobic bacteria, TMC: total microbial counts, US: ultrasounds, UV: ultraviolet, WHO: World Health Organization, WPL: water-assisted pulsed light, YMC: yeasts and moulds count.

Abstract

Strawberries are one of the most important fruits in the Mediterranean diet and have been widely investigated for their nutritional and nutraceutical properties. Concern about the safety of fresh and processed strawberries has increased in recent years due to the emergence of several outbreaks of foodborne pathogens linked to their consumption. The use of chlorine as a disinfectant has been identified as a concern due to public health issues and limited efficacy at removing contamination, and preventing cross-contamination. This has led to the development of novel alternatives to chlorine disinfection and thermal treatments, which include, among others, the use of organic acids, high pressure processing, intense pulsed light, or pulsed electric fields. These technologies do not generally affect the nutritional and organoleptic properties of the product and some of these have been reported to stimulate the production of valuable compounds in strawberries and to improve their overall quality.

Keywords: thermal processing, microbial decontamination, non-thermal processing, chemical decontamination, strawberry, processed fruits

1. Introduction

Different organizations including the World Health Organization (WHO) and the European Food Safety Authority (EFSA), as well as governments worldwide, recommend daily consumption of fruit and vegetables as this has been linked to many positive health outcomes (Giampieri, Alvarez-Suarez, & Battino, 2014; Giampieri et al., 2015; Giampieri, Tulipani, Alvarez-Suarez, Quiles, Mezzetti, & Battino, 2012). However, despite the well-known benefits derived from consuming raw and minimally processed fruit and vegetables, safety is still an issue of concern (Artes & Allende, 2014). The concern about microbiological safety of fresh, minimally processed, or frozen fruit has increased in recent years due to the emergence of several outbreaks of foodborne pathogens linked to their consumption and to the presence of chemical contaminants such as pesticides. One of the most recent outbreaks occurred during the last trimester of 2012, when a norovirus (NoV) gastroenteritis outbreak affected over 11,000 people in Germany. Many more people could have been infected as the outbreak vehicle, frozen strawberries imported from China, was identified within a week leading to a timely recall and preventing more than half of the product reaching the market (Bernard et al., 2014).

The strawberry belongs to the genus *Fragaria* in the Rosaceae. Strawberry consumption has been linked to reduced cholesterol (Basu, Betts, Nguyen, Newman, Fu, & Lyons, 2014; Basu, Nguyen, Betts, & Lyons, 2014; Zunino, et al., 2012) and *in vivo* antioxidant activities (Alvarez-Suarez, et al., 2014; Bialasiewicz et al., 2014). Strawberries are one of the most common and important fruits in the Mediterranean diet and one of the most investigated fruits because of their nutritional and nutraceutical properties (Mezzetti et al., 2014). However, in 2014, the Panel on Biological Hazards of EFSA published a scientific opinion about the risk of *Salmonella* and NoV in berries, including strawberries (EFSA, 2014). EFSA Panel on Biological Hazards concluded that cross-contamination, poor hygiene, or contamination from food handlers, together with the use of contaminated washing water were the main health risks and considered it a priority to carry out more research on decontamination treatments effective against all relevant microbiological hazards including *Salmonella* and NoV in strawberries.

One of the current problems in the food industry is related to the use of chlorine as a disinfectant, which has been identified as a concern due to public health issues and has already been prohibited in some European countries including Belgium, Denmark, Germany, and the Netherlands (Meireles, Giaouris, & Simões, 2016). Limited efficacy at removing contamination and preventing cross-contamination, sequestering of chlorine, and residual odour traits are additional limitations of using chlorine for the disinfection of fruit. As a result, chemical and physical strategies which are environmentally friendly and safe have been developed for the disinfection of fruit and vegetables in the food industry. These include the use of novel chemical strategies, which can be liquids such as electrolyzed oxidizing water (EOW) (Rahman, Park, Song, Al-Harbi, & Oh, 2012) or organic acids (van de Velde, Güemes, & Pirovani, 2014) or gases such as ozone (Brodowska, Nowak, & Śmigielski, 2017), chlorine dioxide (Aday & Caner, 2014) , or ethanol vapour (Li, et al., 2018). Physical strategies currently being utilized or studied include high pressure processing (HPP) (Kim, Gil, Kim, & Cho, 2017) or intense pulsed light (IPL) processing (Duarte-Molina, Gómez, Castro, & Alzamora, 2016). Some of these strategies are of special interest as previous studies have suggested that their use in the food industry has potential applications beyond microbial decontamination and could improve organoleptic as well as nutritional attributes of fruit- and vegetable-based products (Cao, Huang, & Chen, 2017; Islam et al., 2016; Valdivia-Nájar, Martín-Belloso, & Soliva-Fortuny, 2017; Wu et al., 2017; Xu, Chen, & Wu, 2016). For example, Duarte-Molina, et al. (2016) demonstrated how treatment of strawberries using IPL reduced the incidence of postharvest moulds during cold storage and confirmed, using transmission electron microscopy, a strengthening of the strawberry cell walls induced by IPL stress, which resulted in no softening of the fruit.

The current paper reviews risk mitigation systems for safe and high quality fresh or processed strawberries and discusses how these technologies affect health-promoting phytochemicals found in strawberries.

2. Microorganisms in strawberries: Spoilage and human health risks

Despite the health benefits of strawberry consumption, they are generally eaten raw and represent a potential risk for consumers. Fresh and minimally processed fruits are naturally contaminated by diverse microorganisms through different sources, including the field environment, postharvest handling, and processing (Beuchat, 1996). These microbial contaminants could be responsible for the microbial spoilage of strawberries and potential human pathogens have been identified in strawberries. Therefore, their control or elimination is of key importance in order to commercialize safe and healthy products.

2.1 Spoilage microorganisms

Microorganisms that cause spoilage of strawberries and other fruit represent a huge problem for food processors (Petruzzi, Corbo, Sinigaglia, & Bevilacqua, 2017). Indeed, strawberry spoilage losses can be as high as 40 % (Luksiene, & Brovko, 2013). Different strawberry varieties provide diverse ecological niches to microorganisms. The presence, variety, and number of microorganisms also depends on parameters including agronomic practices, geography, weather, harvest, transport, and further handling and processing (Ramos, Miller, Brandão, Teixeira, & Silva, 2013). However, some mould and bacteria species are more often identified in the surface of strawberries and can be considered as the main microorganisms responsible for spoilage. Grey mould caused by *Botrytis cinerea* is the principal fungal decay in strawberries and the main contributor to overall postharvest losses (Kader, 1991). Hashmi, East, Palmer, and Heyes (2013) and Tournas, and Katsoudas (2005) identified both, *B. cinerea* and *Rhizopus stolonifer* as main spoilage microorganisms in strawberries. *B. cinerea* has been also identified in strawberries grown in varied climates such as, Germany (Leroch, Plesken, Weber, Kauff, Scalliet, & Hahn, 2013), Turkey (Ilhan & Karabulut, 2013), and Brazil (Costa, Rangel, Morandi, & Bettiol, 2013). *Alternaria alternata* is together with *B. cinerea* the most dominant mould in strawberries, which produces a toxin responsible for postharvest black rot (Zhang, Sun, Yang, Chen, Li, & Zhang, 2015). Other known fungal species include those reported by Wei, Guo, and Lei (2017) who identified *Mucor fragilis*, *Mucor circinelloides*, *Mucor racemosus*, *Rhizomucor variabilis* and *Penicillium* spp. as the main spoilage-causing fungi on the surface of strawberries.

Several yeasts and bacteria have been also reported in strawberry surfaces. For example, Jensen et al. (2013) identified 22 yeast species from 9 genera, of which species from the genera *Candida*, *Cryptococcus*, and *Rhodotorula* were dominant. In the same study, the authors isolated a large number of bacteria including those from the genera *Curtobacterium*, *Serratia*, *Pseudomonas*, *Enterobacter* and *Rahnella*. Previous studies suggested *Pseudomonas*, *Stenotrophomonas*, *Bacillus*, and *Arthrobacter* as the dominating epiphytic bacteria on strawberry plants – including leaves and flowers (Krimm, Abanda-Nkpwatt, Schwab, & Schreiber, 2005). Moreover, de Melo Pereira, Magalhães, Lorenzetii, Souza, and Schwan (2012) identified several bacterial species in strawberries including *Bacillus subtilis*, *Enterobacter ludwigii*, *Lactobacillus plantarum*, *Pantoea punctata*, and *Curtobacterium citreum*.

2.2 Human-pathogenic microorganisms and mycotoxins

As seen before, the epiphytic microbiota of strawberries is diverse. Occasionally, fruits can become contaminated with pathogenic microorganisms while growing, during harvesting, postharvest handling, processing, or during distribution (Beuchat, 1996). Pre-harvest contamination can occur directly or indirectly via animals, insects, water, soil, dirty equipment, and human handling. During harvesting, postharvest handling and processing, microorganisms could reach the product by human handling, dirty equipment, utensils, or containers.

Limited information about the incidence and survival of pathogens in fresh, minimally processed, and frozen strawberries is currently available. Jensen et al. (2013) isolated potential opportunistic bacterial species including *Rahnella aquatilis*, *Hafnia alvei*, *Chromobacterium violaceum* and different *Staphylococcus* species. These bacteria were reported to cause different infectious human diseases. In the same study, the authors detected a number of yeasts that have been associated with infectious diseases including *Cryptococcus neoformans*, *Candida famata*, and *Candida inconspicua*. In addition, Johannessen et al. (2015) did not find *Campylobacter*, *Salmonella,* and shiga-toxin producing *E. coli* (STEC) in Norwegian strawberries. Similarly, neither *Salmonella* nor STEC were detected in samples of strawberries from primary production in Belgium (Delbeke et al., 2015). An *E. coli* O157:H7 outbreak took place in the United States in 2011 with 15 cases, including 2 deaths (Laidler et al., 2013).

Besides pathogenic bacteria, viruses are also of great concern in fresh and processed strawberries (mainly in frozen strawberries). NoV and hepatitis A virus (HAV) are the main foodborne viruses associated with consumption of fresh and frozen berries worldwide (Palumbo, Harris, & Danyluk, 2016). A huge outbreak of NoV linked to consumption of frozen strawberries from China affected nearly 11,000 people in Germany in 2012 (Bernard et al., 2014). In 2016, a multistate outbreak of hepatitis A linked to frozen strawberries affected 143 persons, 56 of them were hospitalized (CDC, 2016). Recently, a foodborne outbreak also caused by HAV subtype 1B in frozen strawberries from Poland was reported in the European Rapid Alert System for Food and Feed (RASFF) portal (RASFF, 2018a), which affected 13 people in Sweden (RASFF, 2018b). Various berries are increasingly being recognized as vehicles for enteric viruses. Indeed, Baert et al. (2011) reported that the prevalence of NoV in soft red fruits was 34.5% (N=29) and 6.7% (N=150) of the samples tested in Belgium and France, respectively. Li, Butot, Zuber, PROFER, and Uyttendaele (2018) analysed 2015 samples of (frozen) berries (including strawberries) for the presence of HAV, NoV GI and GII. Results demonstrated that 7 of the berry samples were positive for virus (0.3%). In the case of strawberries, 1 out of 918 samples contained NoV GII and 1 was positive for HAV (Li et al., 2018). Macori et al. (2018) evaluated the same virus in 75 berry samples from primary production but the survey did not include strawberries. No viruses were found.

Some of the fungi isolated from strawberries, such as *Penicillium* spp., *Alternaria* spp., and *Rhizopus* spp. are known as potential mycotoxin producers. Little information is known about the presence of mycotoxins in strawberries. In this sense, in strawberries produced in Turkey, Demirci, Arici, and Gumus (2003) found patulin in 8 out of 10 samples analysed (3.2-572 ng/g). On the contrary, Jensen et al. (2013) did not detect mycotoxins in mature strawberries but some strains of *Penicillium expansum* and *Aspergillus niger* isolated from strawberries were able to produce high amount of mycotoxins when incubated in strawberries at 25ºC. Juan, Oueslati, and Mañes (2016) evaluated *Alternaria* mycotoxins in strawberries stored at different temperatures and found alternariol in 42% of samples stored at 22 and in 37% of samples stored at 6 ºC, with concentrations ranging between 26 and 752 ng/g. In addition, alternariol methyl ether was found mainly in stored samples at 6 ºC for more than 28 days and no samples contained tentoxin.

Finally, concerning the prevalence of parasites on strawberries, no studies have been found. However, in the US and Canada, there have been several outbreaks of *Cyclospora cayetanensis* linked to the consumption of raspberries (Palumbo et al., 2014). In 2016, the Netherlands notified a parasitic infestation with microsporidia (presence of *Giardia* parasite) in strawberries from Spain through the RASFF portal (RASFF, 2018c).

Concerning the survival of foodborne pathogens, *E. coli* O157:H7 and *Salmonella* survived but did not grow on the surface of fresh strawberries at 24 and 5 ºC and also survived in frozen strawberries for periods of greater than 1 month (Knudsen, Yamamoto, & Harris, 2001). More recently, Delbeke et al. (2014) assessed the survival of *Salmonella* and *E. coli* O157:H7 on strawberries during a 1-week storage period at refrigerated and ambient temperatures. Results highlighted the importance of avoiding contamination at cultivation and postharvest as washing had only a limited effect and both pathogens survived during storage.

3. Chemical decontamination of strawberries: Effect on microorganisms and quality

As mentioned previously, the use of chlorine as a disinfectant has already been prohibited in some European countries (Meireles et al., 2016). Although several chemical strategies have been studied, those which showed bigger potential for their use for the decontamination of fresh or minimally processed fruit are shown in Figure 1.

Chemical strategies are generally used in whole fruit before their commercialization or before processing in order to reduce the initial microbial load of the strawberries. Acidic EOW is produced by electrolysis of water containing dissolved sodium chloride and has been regarded as a safe and effective antimicrobial agent by the WHO. Over the last decade several studies have reported the bactericidal effect of EOW and demonstrated its effect on a variety of microorganisms in different foods including poultry (Rahman et al., 2012), shrimp (Xie, Sun, Pan, & Zhao, 2012), and lettuce (Forghani et al., 2013). This strategy also showed potential for being used in strawberries (Table 1). Indeed, Guentzel, Callan, Lam, Emmons, and Dunham (2011) suggested that acidic EOW could be used for the disinfection of strawberry plants against *B. cinerea* in the field and Hung, Tilly, and Kim (2010) suggested that acidic EOW was either more or as effective as chlorinated water in killing *E. coli* O157:H7 cells. In that study, the authors observed reductions of *E. coli* O157:H7 ranging between 0.6-0.9, 1.0-1.5, or 1.2-1.5 log colony forming units (CFU)/g when strawberries were dipped in deionized water, EOW, or chlorinated water for 1 or 5 min at 4 ºC. Hung et al. (2010) also observed an effect of temperature on the inactivation studies of *E. coli* O157:H7. Indeed, reductions were significantly lower at 24 ºC when compared to 4 ºC and ranged between 0.3-0.9, 0.6-1.3, and 1.0-1.4 log CFU/g when dipped in deionized water, acidic EOW, or chlorinated water, respectively. One of the main advantages of chemical treatments is that they can be used alone or in combination with physical treatments such as ultrasounds (US) or water-assisted ultraviolet (UV) irradiation, generally obtaining synergistic or additive effects. However, these effects need to be studied for each food matrix and treatment combinations as combining physical and chemical strategies can also result in antagonist effects.

Ozone, a powerful oxidant, has emerged as one of the most promising chemical methods for the preservation of food products and it is highly suitable for fruit and vegetables including strawberries (Tzortzakis & Chrysargyris, 2017). Table 1 lists recent studies which evaluated the effect of ozone in gas or aqueous phase for disinfecting and extending the shelf life of strawberries. For example, Alexandre, Brandão, and Silva (2012) assessed the effect of ozone in aqueous solution at a concentration of 0.3 ppm on the microbial loads and quality attributes of fresh strawberries. Ozone treatment, which was compared to other physical and chemical strategies, provided the best results in terms of reductions of microbial loads, namely total mesophiles, and yeasts and moulds counts (YMC), when samples were kept at room temperature and did not affect the overall quality of the strawberries. Treatment conditions are of key importance and need to be calculated for each product as ozone can affect the quality of fresh strawberries (Aday & Caner, 2014; Aday, Büyükcan, Temizkan, & Caner, 2014). Ozone treatments can be used alone or in combination with other novel strategies such as sonication. Indeed, Aday and Caner (2014) evaluated the effect of ozone at a concentration of 0.075 ppm alone or in combination with US and demonstrated that although ozone alone significantly reduced the physical deterioration and spoilage of strawberries, increasing their shelf life, a combination of both strategies was more effective. For example, the initial a^* value of the strawberries in that study was 34.3 and decreased to 30.1, 31.2 and 32.3 during a 4-week storage period in samples left untreated, treated with 0.075 ppm of ozone alone or combined with US, respectively. The authors of that study suggested that the combination of ozone and US maintained the phenolic content and inhibited the colour change chemically better when compared to ozone or US alone. Ozone has been also used for the removal of fungicides and insecticides. Indeed, Lozowicka, Jankowska, Hrynko, and Kaczynski (2015) obtained reductions ranging from 36.1 to 75.1% in the concentration of 16 pesticides (10 fungicides and 6 insecticides) after immersion of strawberries in ozone, in aqueous phase (20 ºC, 1 mg/L), for processing times ranging from 1 to 5 min. Even higher reductions were reported by Heleno, De Queiroz, Neves, Freitas, Faroni, and De Oliveira (2014) who obtained a 95% reduction in the concentration of difenoconazole residue after exposure of contaminated strawberries to ozone gas at concentrations ranging from 0.0 to 0.8 mg/L for 1 h.

Organic acids or chlorine dioxide have also been evaluated as potential substitutes for sodium hypochlorite (Table 1). For example, Aday, Buyukcan, and Caner (2013) studied the effect of chlorine dioxide in combination with modified atmospheric packaging (MAP) at concentrations of 3, 6, and 9 ppm on the overall quality of fresh strawberries and demonstrated a significant reduction on the respiration rate as well as an increase of the shelf life. Although results obtained so far suggest chlorine dioxide as an excellent alternative to chlorine, some contradictory results have been published. For example, Arango, Rubino, Auras, Gillett, Schilder, and Grzesiak (2016) treated strawberries with continuously generated chlorine dioxide gas at concentrations ranging from 0.01 to 5.00 mg/L and durations ranging from 7 to 1000 min. The authors observed that treatments had a minimal effect at delaying the growth rate of *B. cinerea* at 4 or 22 ºC and suggested that chlorine dioxide treatments were not enough to extend the shelf life of strawberries. Although the antimicrobial effect of peracetic acid (PAA) is well known, a number of studies suggested that treatment using PAA, depending on the concentration, could result in a loss of quality in strawberries. For example, van de Velde, Piagentini, Güemes, and Pirovani (2013) observed reductions of 30 and 37% in the total anthocyanin and ascorbic acid content, respectively when dipping two varieties of strawberries in 80 mg/L for 2 min. In order to optimize the disinfection process, van de Velde, et al. (2014) developed a model to evaluate the microbial count reduction under specific PAA concentration, temperature, and treatment time of fresh-cut strawberries. Treatment conditions obtained to maximize the total microbial count (TMC) reduction were 100 ppm at 24 °C for 50 s. However, those conditions resulted in the appearance of off-odours and off-flavours as well as low retention of anthocyanins and ascorbic acid. In that same study, treatment conditions obtained to maximize anthocyanin and ascorbic acid retention, with a 2-log CFU/g reduction in the TMC, were 20 ppm at 18 °C for 52 s. The authors of that study suggested the latter conditions to fresh-cut strawberries disinfection because of acceptable TMC reductions together with higher retention of total anthocyanins and ascorbic acid as well as better sensory attributes and economic convenience.

Overall, based on the studies published to date, reductions of microorganisms obtained by different chemical alternatives are not so different from those obtained with sodium hypochlorite. It has to be taken into account that long processing times that have been studied are not feasible for practical application. PAA is easy to use and control, while ozone has some concerns due to legal limits in ambient and some problems to control its concentration in washings. However, ozone has the advantage of availability of generators and lack of disinfection by products. Chlorine dioxide has some persisting concerns over chlorite residues and some bleaching action that could affect product quality.

4. Physical decontamination of strawberries and strawberry-derived products

4.1 Effect of thermal and non-thermal strategies on microorganisms

Physical methods for food preservation are those that utilize physical treatments to inhibit, destroy, or remove undesirable microorganisms without involving antimicrobial additives. Table 2 lists previous works which studied novel physical technologies which can be used to improve quality and to reduce the microbial load of strawberries and strawberry-derived products. These can be divided into those that involve heating and novel non-thermal treatments such as HPP, pulsed electric fields (PEFs), cold atmospheric plasma (CAP), or those shown in Figure 1. Although some of these techniques can cause a moderate temperature elevation in the food matrix, the increase in temperature is not their main mechanism of action. These technologies can also be divided into those that can be used on processed strawberry-derived products such as jams, juices, or purees and those which aim to be used on fresh and minimally processed strawberries.

4.1.1 Thermal processing

The basic purpose of thermal processing of foods is to reduce microbial and enzymatic activity and to produce physical and chemical changes to make food meet a quality standard. Heat processing is most commonly used in the fruit processing industry to ensure safety and stability of juices, nectars, purées, and jams. The heating process should affect the properties of the product as little as possible keeping prices low.

Over the last decades a number of novel heating technologies with shorter start-up times, faster heating, greater energy efficiency, small footprint, and improved organoleptic and nutritional quality of the end product have been developed and these include microwave processing and ohmic heating. Microwave heating has gained special interest in food processing due to its ability to obtain high temperatures, reduce processing time, and result in a more uniform heating (Stratakos, Delgado-Pando, Linton, Patterson, & Koidis, 2015). This technology has been efficiently used for the treatment and optimization of decontamination strategies of strawberries and strawberry-based products.

For fresh and minimally processed strawberries, mild heat treatments are more appropriate, due to the changes that high temperatures could cause to the fruit. Indeed, Fang, Pengyu, and Xiaohu (2013) suggested that a combination of hot water (40 °C, 5 min), microwave processing, and the use of a composite coating on strawberries was the best processing option to prolong shelf life. Microwaves have been also used alone or in combination with vacuum as a novel method for drying strawberries obtaining high quality products in terms of appearance, colour, and texture (Bórquez, Melo, & Saavedra, 2015) and extending the shelf life of the dried product (Bruijn et al., 2016).

4.1.2 Non-thermal technologies

The use of heat through thermal processing operations including blanching, pasteurization, and sterilization is still being used as a common practice by food manufacturers. However, as indicated before, these technologies are either undesirable or cannot be used for certain foods such as fresh produce.

HPP is an innovative but industrially consolidated technology for processing a wide range of food products and represents an ideal alternative to heat processing. One of the main advantages of this technology is extending the shelf life while retaining the sensory characteristics of fresh foods. Disadvantages of this technology include that it cannot operate in continuous mode and that it cannot be used on whole fruit without modifying quality attributes such as texture. However, several studies have demonstrated the antimicrobial potential of HPP on strawberries. For example, Marszałek, Mitek, and Skąpska (2015b) showed how HPP at 500 MPa reduced the CFU of YMC in strawberry puree from 4.6- and 3.8-log CFU/g to less than 1-log CFU/g at both, 0 and 50 °C. In that same study, treatment at 200 MPa also resulted in lower yeast and mould counts with reductions of 2.6- and 0.5-log CFU/g, respectively. Hsu, Sheen, Sites, Huang, and Wu (2014) obtained a reduction of *E. coli* O157:H7 greater than 5-log CFU/g after treatment of strawberry puree at 250 and 350 MPa for 5-30 min at 10 °C. At those conditions, the *E. coli* O157:H7 counts were below the detection limit (1.5-log CFU/g). Similar results were obtained by Huang, Ye, and Chen (2013), who eliminated *E. coli* O157:H7 and *Salmonella* spp. from strawberry puree after processing at 450 MPa during 2 min at 21 °C. Research has focused mainly on how HPP causes bacterial and fungal inactivation. However, HPP can even cause damage to viruses by damaging the virus envelope preventing their particles binding to cells or even by a complete dissociation of the virus particles (Considine, Kelly, Fitzgerald, Hill, & Sleator, 2008). Huang, Li, Huang, and Chen (2014) recently suggested that HPP of strawberries and strawberry purée was efficient in inactivating murine norovirus 1 (MNV-1). In that study, MNV-1 was very resistant to pressure under the dry state condition, but became sensitive to pressure under the wet state condition and the efficacy of HPP inactivation increased with decreasing initial sample temperature. A treatment time of 2 min was needed to achieve a 4.3 log reduction of MNV-1 in puree at 350 MPa, while 4 min were needed to obtain the same level of reduction at 300 MPa. Inactivation curves were almost linear with R^2 value of 0.99. In addition, the calculated D values for whole strawberries and strawberry puree were similar and calculated as 0.86 min. In that same study, after processing, samples were frozen and stored at -20 ºC for 28 days and the authors observed additional 0.4 and 0.6 log reductions of MNV-1 for samples treated at 300 and 350 MPa, respectively. Similar results were obtained by Kovač, Diez-Valcarce, Raspor, Hernández, and Rodríguez-Lázaro (2012) in strawberry puree.

Table 2 describes major findings on the potential of PEFs for being used to control microorganisms in processed strawberry-derived products. Microbial inactivation by PEFs occurs due to the electrical breakdown of cell membranes caused by the build-up of electrical charges at the cell membrane that ends with the cell membrane disruption (Odriozola-Serrano, Aguiló-Aguayo, Soliva-Fortuny, & Martín-Belloso, 2013). PEFs consist of very short pulses (μs) of electricity to liquid foods placed between two electrodes. Therefore, this technology could be used for decontamination of strawberry juices or purees and not for the whole fruit. Mosqueda-Melgar, Raybaudi-Massilia, and Martín-Belloso (2008) studied the effect of PEFs on the *S. enteritidis* and *E. coli* O157:H7 populations inoculated in strawberry juice and concluded that microbial reductions increased when treatment time was higher, showing a logarithmic behaviour. Maximum bacterial inactivation was calculated as 4.43- and 5.46-log CFU/g for *S. enteriditis* and *E. coli* O157:H7, respectively and were obtained operating at 1700 μs and 100 Hz. In a more recent study, Gurtler, Bailey, Geveke, and Zhang (2011) obtained inactivations of *E. coli* O157:H7 of 2.86-, 3.12-, and 3.79-log CFU/g at temperatures of 45, 50, and 55 °C, respectively. The authors of this study also demonstrated that the preservatives sodium benzoate, potassium sorbate, and citric acid induced sub-lethal injury and enhanced PEF inactivation of *E. coli* O157:H7 and non-pathogenic *E. coli* in strawberry juice.

Methods based on the antimicrobial effects of UV irradiation have also been extensively studied. This technology can be used for both fresh and processed strawberries. However, turbidity, suspended solids, and absorbing compounds are key parameters which affect the potential of this technology to disinfect liquid products (Selma, Allende, López-Gálvez, Conesa, & Gil, 2008) and because of the intense colour of strawberries, this could be a disadvantage. However, Bhat, and Stamminger (2015) observed a 2-log reduction in the total aerobic bacteria (TAB) plate counts as well as in total YMC after exposure of strawberry juice to UV radiation (254 nm) for 15-60 min. Similar results were reported by Keyser, Műller, Cilliers, Nel, and Gouws (2008) after UV radiation of strawberry juice as described in Table 2. UV light inactivation could also be used for the inactivation of NoV as previous studies demonstrated that this technology was efficient in inactivating HAV, Aichi virus A, and feline calicivirus on whole strawberries (Fino & Kniel, 2008). Water-assisted pulsed light (WPL) treatments have also been used for the inactivation of MNV-1. Indeed, Huang and Chen (2015) studied the effect of WPL in combination with 1% hydrogen peroxide or 100 ppm sodium dodecyl sulphate (SDS) on the inactivation of *E. coli* O157:H7, *Salmonella*, and MNV-1 in fresh strawberries. The authors of that study reported a reduction in the *E. coli* O157:H7 and *Salmonella* counts of 2.4- and 4.5-log CFU/g after WPL treatment for 60 s. Photosensitization is a novel non-thermal and environmentally friendly technology which involves the administration of photoactive compounds and visible light. This strategy can also be utilized for microbial decontamination of strawberries. Indeed, Luksiene and Paskeviciute (2011) studied the potential of chlorophyllin-based photosensitization to control microbial contamination of strawberries. Strawberries were inoculated with *Listeria monocytogenes*, soaked in 1 mM chlorophyllin for 5 min and illuminated for 30 min with visible light. The authors of that study observed 86 and 97% inhibition in naturally occurring yeasts/moulds and mesophiles, respectively and the shelf life of the strawberries was extended by 2 days.

US is one of the newest non-thermal technologies to extend the shelf life of fruit. The efficacy of this strategy depends on several parameters including wave frequency, power, and treatment time (de São José, de Andrade, Ramos, Vanetti, Stringheta, & Chaves, 2014). This strategy has been studied as an alternative to prevent microbial spoilage of both fresh strawberries and processed strawberry-derived products. Table 2 lists several studies which used this technology alone or in combination with chemical sanitizers over the last 5 years. For example, Aday, Temizkan, Büyükcan, and Caner (2013) evaluated the effect of different US powers (30, 60, and 90 W) during 5 or 10 min on the quality of fresh strawberries. Results demonstrated a significant decrease in the appearance of mould during storage and no differences were observed between samples treated at 30, 60, or 90 W. Similar results were obtained by Gani et al. (2016), who demonstrated that the bacterial count decreased from 5.9- to 3.9-log CFU/g while the yeast and mould count decreased from 4.8- to 3.5-log CFU/g after US processing (33 kHz, 60W) of fresh strawberries. Antimicrobial effect of US has been attributed to two main causes, cavitation and the formation of free radicals which result in thinning and disruption of cell wall structures, pore formation and cell membrane disruption and DNA injuries with produce breakages and fragmentation (de São José et al., 2014). This technology can be used in combination with other chemical or physical strategies such as heat, in a process known as thermosonication (São José & Vanetti, 2015).

The lethal capabilities of CAP have now been amply studied on a wide variety of microorganisms including biofilm formers and bacterial spores. Although this technology is relatively new and there are limited numbers of reports based on CAP decontamination of fresh produce, Table 2 lists examples of studies which evaluated the potential of this technology for the disinfection of strawberry. Overall, antimicrobial efficacy of this technology varies depending on several factors including the system used to produce the plasma, gas composition, and electrode configuration as well as type of bacteria and substrate (Ziuzina, Patil, Cullen, Keener, & Bourke, 2014).

4.2. Thermal and non-thermal processing technologies and their effect on fruit quality

Blanching, pasteurization, and sterilization can degrade nutritionally important phytochemicals (Lafarga, Bobo, Viñas, Collazo, & Aguiló-Aguayo, 2018; Nayak, Liu, & Tang, 2015). Although novel non-thermal technologies can also have an effect on the concentration of health-promoting compounds such as anthocyanins, overall, results obtained so far suggest that if a loss of phytochemicals is produced after non-thermal processing, this would be smaller compared to that obtained after a traditional thermal treatment (Marszałek, et al., 2015b; Marszałek, Woźniak, Kruszewski, & Skąpska, 2017). The phytochemical content and quality of fresh and processed strawberries depends on several factors including season, maturity, variety, and processing conditions including treatment intensity and duration (Ban, et al., 2018; Oszmiański, Lachowicz, Gorzelany, & Matłok, 2018; Salvador, Rocha, & Silvestre, 2015; Šamec, Maretić, Lugarić, Mešić, Salopek-Sondi, & Duralija, 2016; Xie, et al., 2015). This has to be calculated for each process independently and needs to be considered when calculating the dietary intake of these compounds from processed strawberries and strawberry-based products.

4.2.1 Thermal processing

According to Patras, Brunton, O'Donnell, and Tiwari (2010), it is not possible to predict the effect of thermal treatment on retention of bioactive compounds, and it is necessary to evaluate each case individually. In addition, besides some mild treatments which do not significantly affect the texture of whole fruit, thermal processes are generally used for juices, jams, or purees. Previous studies suggested that microwave heating might change the phytochemical content and the overall quality of foods to a lesser extent as opposed to conventional heating. For example, Marszałek, Mitek, and Skąpska (2015a) compared the effect of conventional heating and heating using a continuous flow microwave on the safety, shelf life, and quality of strawberry purée. Continuous microwave treatment (2.45 GHz, 63 A, 20 kW) at 80 or 120 °C during 7 or 10 s resulted in being significantly less destructive for phenolic compounds, flavonoids, anthocyanins, and vitamin C when compared to the conventional thermal treatment (90 °C during 15 min). Although some changes in colour were detected, these were barely visible and the overall quality of the purée was not affected. Inactivation of polyphenoloxidase (EC 1.14.18.1; PPO) and peroxidase (EC 1.11.1.7; POD) together with microbial decontamination is one of the main goals of fruit processing. Marszałek, et al. (2015a) did not observe a complete inactivation of PPO and POD after microwave processing of strawberries.

Although the concept of ohmic heating is not new, this technology has recently gained new interest because the products obtained using it are generally of better quality than those obtained using conventional heating technologies (Castro, Teixeira, Salengke, Sastry, & Vicente, 2004). This technology was used for the dehydration of strawberries obtaining beneficial effects on their microstructure and on the kinetics of water loss (Moreno, et al., 2012a) and also on the overall quality and shelf life of the product (Moreno, et al., 2012b).

In addition, as mentioned previously, mild heat treatments can also be used to increase the shelf life of strawberries and strawberry-derived products. Caleb et al. (2016) investigated the impact of mild hot water dipping (35 and 45ºC) during 5 or 10 min on the physicochemical quality (mass loss and transpiration, surface color, texture, total soluble solids, titrated acidity and pH), individual sugars, antioxidant activity, anthocyanin and visual quality of freshly harvested strawberries stored at 4ºC. The microbial quality was not investigated but results showed that hot water treatment at 45 ºC for 5 min had no detrimental effects and best maintained quality attributes of strawberries and prevented incidence of decay.

4.2.2 Non-thermal technologies and their effects on the quality of strawberries

In recent years, consumers have become more aware of the influence of food on health and well-being and there has been a growth in the demand for high quality, minimally processed foods that are both nutritious and tasty. This has led to the development of novel non-thermal technologies which ensure the safety and stability of foods while minimizing the degradation of nutritious and tasty compounds.

Strategies which are used on liquid strawberry-derived products such as juices are mainly HPP and PEFs. Although treatment of strawberries using HPP can improve the sensory quality of products when compared to a conventional thermal processing, HPP can degrade total polyphenols, anthocyanins, and vitamin C in strawberries (Marszałek, et al., 2015b). Verbeyst, Bogaerts, Van der Plancken, Hendrickx, and Van Loey (2013) proposed a model to describe the degradation of ascorbic acid during thermal processing at atmospheric pressure and at 700 MPa and concluded that the combination of HPP with heat enhanced the thermal degradation of ascorbic acid in both aerobic and anaerobic conditions. The authors suggested that the use of HPP could be advantageous on the pasteurization level but not on the sterilization level of strawberries, even if times could be reduced. HPP has been shown to reduce the activity of enzymes including PPO and POD in strawberries previously (Marszałek, et al., 2015b). However, HPP-induced protein denaturation can be reversible depending on several factors including temperature, treatment time, intensity, and also the type of protein (Considine, et al., 2008). Sulaiman and Silva (2013) reported that treatment of strawberries at 600 MPa resulted in high inactivation of PPO, although some residual activity was observed after 15 min.

PEFs showed excellent antimicrobial effects on strawberry juices and purees (Table 2). In addition, Mosqueda-Melgar, Raybaudi-Massilia, and Martín-Belloso (2012) recently reported no differences in the aroma and colour of strawberry juice and PEF processed juice (35 kV/cm for 1,700 µs in bipolar 4 µs pulses at 100 Hz). In addition, although the authors of that study observed a decrease in taste and overall acceptance after processing, the observed decrease was smaller when compared to the one observed after thermal processing at 90 ºC for 1 min. Odriozola-Serrano, Soliva-Fortuny, and Martín-Belloso (2008) observed how PEF treated strawberry juice (35 kV/cm for 1,700 µs in bipolar 4 µs pulses at 100 Hz) maintained higher amounts of polyphenols including anthocyanins, and ellagic and coumaric acid when compared to the thermally treated juice (90 ºC for 1 min). However, the higher content of health-related compounds was not reflected in a higher antioxidant capacity.

UV-C processing of strawberries is thought to be effective not only in extending shelf life and improving organoleptic properties but also in increasing the content of health-promoting phytochemicals. For example, Xie, et al. (2015) studied the effect of UV-C on the antioxidant capacity and phytochemical profiles of three different strawberry cultivars and, although processing did not affect the antioxidant capacity of the fruit, the phytochemical content of the cultivar 'Albion' significantly increased after processing. A recent study carried out by Oviedo-Solís, et al. (2017) reported an *in vitro* increase in the antioxidant activity, attributed to an increase in the content of polyphenols (flavonoids, anthocyanins, fisetin, and pelargonidine), of strawberries after being irradiated with UV-C at 1.2 W/m^2 during 16.5 min. In addition, in that same study, the authors assessed the *in vivo* antioxidant potential of freeze-dried irradiated and non-irradiated strawberries using high fat diet-induced rats and demonstrated how the irradiated strawberries were better than the control, reducing the oxidative damage in brain, probably due to the increased content of flavonoids. However, these results contrast with other studies which suggested a decrease in the antioxidant potential and in the phytochemical content of strawberries after UV-C processing. Indeed, some studies suggested a reduction in the content of ascorbic acid, anthocyanins, and total phenols together with a decrease in antioxidant activity after UV processing (Bhat, et al., 2015). Other quality parameters such as the sugar content or the content of organic acids were not affected after UV-C processing of strawberries either (Xie, et al., 2016). There is no way of predicting these effects of UV processing on the quality of fruit and these need to be assessed for each product independently. Based on the results previously reported and described in the current paper, it seems that the effect of UV-C processing on the levels of bioactive compounds in strawberries depends on several factors which include variety, climate, season, as well as processing parameters such as intensity or duration. However, further studies are needed in order to obtain robust conclusions. Only few studies have assessed the effect of IPL processing on strawberries. Duarte-Molina, et al. (2016) recently observed a cell wall stregthening after processing although weight loss through storage was similar in untreated and treated samples. In that study, IPL treatment delayed the onset of infection of strawberries, which was visually inspected. Moreover, Luksiene, Buchovec, and Viskelis (2013) did not observe any improvement in the organoleptic and nutritional quality of strawberries after IPL treatment, besides a reduction in the microbial load and a 2-day increase of the products shelf life. Further studies are needed in order to assess its potential for improving quality in strawberries. Overall, results reported so far suggest no significant effects on the overall quality besides microbial decontamination. However, further studies are needed in order to assess if this technology could be used to increase quality of fresh or processed strawberries. Photosensitization resulted in increased antioxidant capacity of strawberries previously (Luksiene, et al., 2011).

However, further studies are needed in order to assess the real effect of this technology on the quality of fresh and processed strawberries.

Aday, Temizkan, Büyükcan, and Caner (2013) evaluated the effect of different US powers (20 kHz; 30, 60, or 90 W) during 5 or 10 min on the quality of strawberries. The authors of this study concluded that while US power of 90 W resulted in negative effects on the fruits´ quality (reduction in lightness, firmness, and red hue), power levels between 30 and 60 W improved colour and firmness retention and enhanced shelf life. Similar results were obtained by Gani, et al. (2016), who demonstrated that US (33 kHz, 60W) enhanced antioxidant activity and facilitated better retention of pH, colour, and texture. Tomadoni, Cassani, Viacava, Moreira, and Ponce (2017) recently obtained an increase in both, polyphenol content and antioxidant activity after sonication of strawberry juice at 40 kHz for 10 or 30 min, when compared to thermally treated juice at 90 °C for 1 min. Similar results were obtained by Bhat and Goh (2017) who obtained a significant enhancement in bioactive compounds after processing for 30 min. In a different study, Sulaiman, Soo, Farid, and Silva (2015) inactivated PPO in strawberries by thermosonication, the combination of US and heat, during 10 min at 32 °C. Although the quality attributes of the samples were not assessed, much lower processing temperatures were needed when compared to thermal processing alone, and the authors suggested that a potentially better fruit quality could be obtained. US shows a big potential for being used in the food industry not only for enzymatic and microbial inactivation but also for the extraction of valuable phytochemicals which could be further included into foods as functional ingredients (Sun, Zhai, Zhang, Qiu, Ou, & Bai, 2014).

Although some studies have suggested an increase of the anthocyanin content of some fruits after treatment using CAP (Kovačević, Putnik, Dragović-Uzelac, Pedisić, Režek

Jambrak, & Herceg, 2016), so far, no significant effects have been observed on the nutritional quality of processed strawberries. This technology would allow to retain the quality of fresh strawberries while it significantly improves their shelf life. For example, Misra, et al. (2014a) observed no adverse effects on respiratory rates, texture, or colour of strawberries after processing and Misra, et al. (2014b), who evaluated the use of CAP induced in MAP gases for fresh strawberries in a closed package, observed how besides extending shelf life, strawberries treated and stored in a high oxygen gas mixture showed more favourable respiration rates and a higher firmness than the control over a 24 h period.

5. Conclusions

A large number of chemical and physical alternatives to chlorine have been reported over the last couple of decades. Some of these showed promising results and could be as efficient as chlorine, or even more, in eliminating microorganisms from the surface of strawberries. Overall, the use of chemical and physical non-thermal strategies seems to result in better retention of antioxidants and phytochemicals in strawberries when compared to conventional thermal treatments. These technologies can be divided into two groups, based on their use in fresh or minimally processed strawberries or in liquid strawberry-derived products such as purees, nectars, or juices. From those technologies which can be used on fresh or minimally processed samples, IPL and UV-C irradiation showed the best results as besides microbial inactivation, several reports highlighted an increase in the nutritional value of strawberries after processing. In addition, PEFs showed promising results and could be an alternative to thermal pasteurization of strawberry-derived products. From those chemical strategies studied over the last years, ozone either in the gaseous or liquid phase showed microbial reductions (including human pathogens) comparable to those obtained using chlorine. Acidic EOW and PAA treatments also resulted in promising results and show potential for being used as substitutes of chlorine in the food industry. Most of these technologies are environmentally friendly, economically viable, are accepted by consumers, and show potential for their use during the industrial production of safe, nutritious, and tasty strawberry-based products.

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Conflict of interests

The authors declare no conflict of interests

Figures

Figure 1. Summary of alternatives to chlorine and conventional thermal pasteurization which could be used to improve safety, quality, and shelf life of strawberries

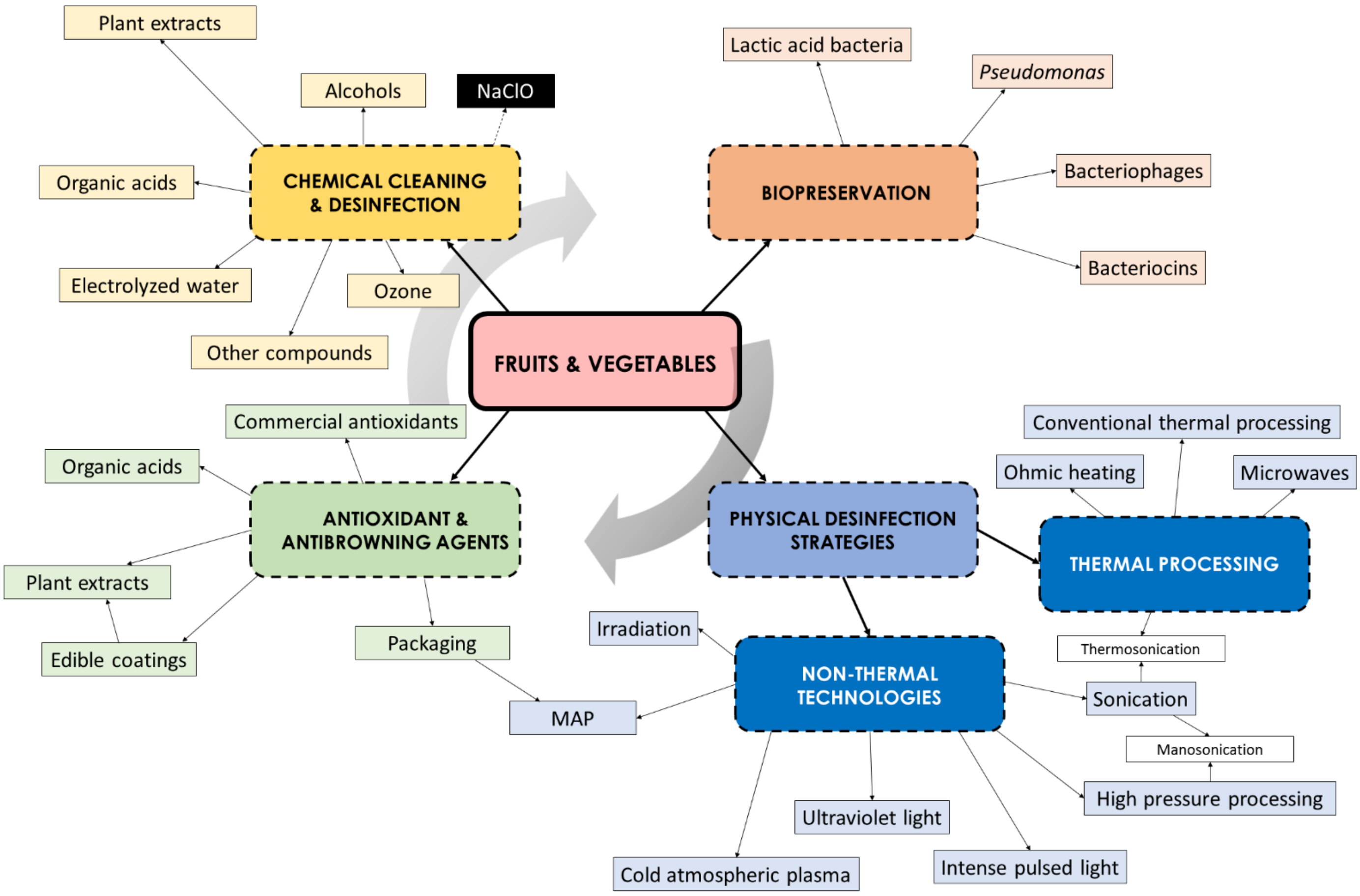

Table 1. Overview of chemical strategies applied for the control of microorganisms on strawberries and strawberry-derived products.

Chemicals evaluated	Treatment conditions	Microorganisms studied	Food matrix	Main outcomes	Reference
Acidic EOW and chlorinated water	Acidic EOW: 23 and 55 mg/L of residual chlorine Chlorinated water: 100 mg/L of residual chlorine Exposure time: 1 or 5 min	*E. coli* O157:H7	Inoculated strawberries and broccoli	Inactivation of *E. coli* O157:H7 was temperature and time dependent. Increasing soaking times from 1 to 5 min reduced populations of the pathogen by 0.1- to 0.8-log CFU/g regardless of treatment solution.	(Hung et al., 2010)
Acidic EOW	Available chlorine concentration: 34.3 mg/mL	TAB and YMC	Fresh fruits including strawberries	Treatment using acidic EOW resulted in approximately 0.9-log reductions for both TAB and YMC.	(Ding, Ge, Shi, Xu, Jones, & Liu, 2015)
EOW	Available chlorine concentration: 39.24 and 68.13 ppm Exposure time: 1, 5, 10 min	Mesophilic aerobic bacteria and YMC in un-inoculated samples. *E. coli* O157:H7 and *L.monocytogenes* artificially inoculated	Strawberries	Aerobic mesophiles were reduced more than 2-log CFU/g after washing for 10 or 15 min in EOW prepared from 0.10% (w/v) NaCl solution (68.1 ppm). NaOCl and EOW solutions demonstrated a comparable antimicrobial effect against *L. Monocytogenes* and *E. coli* O157:H7.	(Udompijitkul, Daeschel, & Zhao, 2007)
Ozone, chlorinated water, and hydrogen peroxide	Ozone: 0.3 ppm Chlorine: 200 µg/mL Hydrogen peroxide: 1 and 5% Exposure time: 2 min	Total mesophiles, YMC	Fresh strawberries	Strawberries washed with hydrogen peroxide solutions at 5 and 1% had the highest total mesophiles reduction measured as 2.2- and 1.5-log unit reductions, respectively. On average, a 1.2-log unit reductions occurred when samples were washed with aqueous ozone solutions. However, ozone treatment maintained the lowest total mesophiles load after storage for 4 days at 4 °C.	(Alexandre et al., 2012)
Ozone	Concentration: 0.075, 0.150, and 0.250 ppm	Moulds	Fresh strawberries	All ozone treatments prevented mould growth during storage. However, the 0.250 ppm ozone treatment caused	(Aday, Büyükcan, Temizkan, & Caner,

	Exposure time: 2 and 5 min			loss of strawberry quality due to high ozone concentration. Ozone could be applied to extend the shelf life of strawberries by at least 3 weeks under refrigerated conditions.	2014)
Ozone	(i) Continuous ozone flow (5%) for 2-64 min; (ii) Pressurizer ozone (83 kPa) for 2-64 min; (iii) Continuous ozone for 64 min followed by pressurized ozone for 64 min; (iv) vacuum followed by 64 min of pressurized ozone.	*E. coli* O157:H7 and *S. enterica*	Inoculated strawberries	Continuous ozone followed by pressurized ozone showed the highest reductions of *S. enterica* (2.6-log reductions) and *E. coli* O157 H:7 (2.9-log reductions). Continuous ozone flow, pressurized ozone and vacuum followed by pressurized ozone treatments after 64 min reduced *S. enterica* population by 0.9-, 2.2- and 1.7-log units, and *E. coli* O157H:7 by 1.8-, 2.3- and 0.9-log reductions, respectively.	(Bialka & Demirci, 2007)
Ozone	Gaseous ozone at 6% W/w, 10, 20, 30, 40 min	MNV-1 and Tulane virus	Inoculated lettuce/strawberries	Gaseous ozone efficacy was dose and time dependent. After 40 min, ozone completely inactivated NoV in liquid media and reduced it on strawberries surfaces	(Predmore, Sanglay, Li, & Lee, 2015)
Chlorine dioxide	Concentration: 10 mg/L Exposure time: 3 min	*E. coli* O157:H7, *S. enterica*, *L. monocytogenes*, TAB, and YMC	Inoculated tomatoes, cantaloupes, and strawberries	Nearly a 5-log CFU/cm^2 *Salmonella* reduction was found on tomatoes, cantaloupe, and strawberries, while a 3-log CFU/cm^2 reduction was observed for *E. coli* and *Listeria* on all produce surfaces. *E. coli* and *Listeria* appeared to be more resistant to chlorine dioxide as compared to *Salmonella* spp.	(Trinetta, Linton, & Morgan, 2013)
Chlorine dioxide	Concentration: 0.5-5.0 mg/L)	*E. coli* O157:H7, *L. monocytogenes*, *S. enterica*,	Artificially inoculated strawberries	Approximately a 4.3-4.7-log CFU/strawberry of all examined bacteria was achieved by treatment with 5 mg/L ClO_2 for 10 min	(Mahmoud, Bhagat, & Linton, 2007)
PAA	Concentration: 0-100 mg/mL Exposure time: 10-120 s Temperature: 4-40 ºC	TMC	Fresh strawberries	After modelling the results, two optimization scenarios were studied: OP1 (100 mg/L, 50 s, and 24 °C) and OP2 (20 mg/L, 52 s, and 18 °C). OP1 and OP2 reached reductions of 1.8- and 0.8-log CFU/g of microbial count, respectively. OP2 conditions resulted in better sensory attributes and the economic convenience of lesser PAA	(van de Velde, et al., 2014)

				consumption.	
Strawberry-flavoured vinegar - Acetic acid	Concentration: 0.000-0.225%	*B. cinerea*	Inoculated strawberries	Baby corn fermented vinegar containing 0.225% acetic acid completely inhibited the growth of *B. cinerea*. Shelf life at 4 °C of strawberries sprayed with vapour of strawberry-flavoured vinegar was extended to 7 days while that of fruit exposed to liquid vinegar was extended to 11 days.	(Krusong, Jindaprasert, Laosinwattana, & Teerarak, 2015)
Acetic acid vapour	(i) Application at 2 mg/L for 30 min once, twice, or three times; (ii) 4 mg/L for 30 min; (iii) 6 mg/mL for 30 min	*B. cinerea* and microflora	Fresh strawberries	Triple fumigation with 2 mg/L acetic acid vapour was found to be most effective treatment resulting in a 56 % reduction of decay. Alternatively, a single treatment with 6 mg/L AA vapour resulted in a 44 % reduction of decay. The aerobic mesophilic bacteria plate count was only slightly affected by fumigation. Applying 3 mg/L acetic acid vapour for 30 min reduced mould counts from $2.0 \cdot 10^5$ CFU/g to less than 10^3 CFU/g.	(Hassenberg, Geyer, & Herppich, 2010)
PAA and hydrogen peroxide	Sanitizer mixture of PAA at 5% and hydrogen peroxide at 20%. Concentration: 3.4-116.6 µL sanitizer/L air chamber. Treatment time: 5.7-69.3 min	Total mesophilic microorganisms and YMC	Strawberries	Treatment with 116.6 µg/L PAA plus hydroxen peroxide for 37.5 min showed significantly highest efficacy reducing mesophilic microorganisms by 3.0-log units and YMC by 1.3-log reductions. Similarly, treatment with 100 µg/L PAA plus hydrogen peroxide for 60 min reached reductions of 2.7- and 3.1-log units to mesophilic microorganisms and YMC, respectively. Optimal fogging conditions achieved were 10.1 mL sanitizer/L air chamber and 29.6 min	(van de Velde, Vaccari, Piagentini, & Pirovani, 2016)
SDS and hydrogen peroxide	Concentration: 1% hydrogen peroxide and 100 ppm SDS Treatment time: 1 min	*E. coli* O157:H7, *Salmonella*, and MNV-1	Artificially inoculated fresh strawberries	Treatment with hydrogen peroxide and SDS reduced *E. coli* O157:H7 by 1.9- and 1.6-log CFU/g, respectively.	(Huang et al., 2015)
Chlorinated water and levulinic acid (LVA) plus	(i) Chlorinated water at 50 ppm; (ii) 0.5% LVA plus 0.5% SDS; (iii) 5%	*Enterococcus faecium*, *L. monocytogenes*, *S. enterica*, *E. coli* O157:H7, *E. coli*, MNV-	Fresh strawberries	The 50 ppm chlorine wash induced 3.4- and 1.5-log reductions for HAV virus and MNV-1, respectively. The tested bacterial strains showed uniform reductions around 1.6-log CFU/mL. The 0.5% LVA plus 0.5% SDS wash	(Zhou et al., 2017)

SDS	LVA plus 2% SDS. Exposure time: 2 min	1 and other viruses.		induced 2.7- and 1.4-log reductions HAV and MNV-1, which were comparable with the reductions induced by chlorine. For bacteria, over 2.0-log reductions were obtained for *E. faecium*, *L monocytogenes* and *Salmonella*, while *E. coli* O157:H7 and *E. coli* showed reductions of 1.9- and 1.8-log CFU/mL. Higher concentration of LVA plus SDS showed no significantly higher reductions.	
Edible coatings containing allyl isothiocyanate (AIT) and lauric arginate ester (LAE)	Micro-emulsions were obtained from a solution consisting of 1% chitosan, 0.5% corn-biofiber gum, and 1–4% AIT or LAE followed by high pressure homogenization.	*S. enterica* and *E. coli* O157:H7	Strawberries	LAE films reduced the cell populations to approximately 4.0-log on strawberries at day 1 and maintained them at this level through day 5. AIT films reduced the populations by 1.0-log at day 1, but continuously reduced the populations to 2.8-log after 5 days.	(Guo, Yadav, & Jin, 2017)
Edible coatings containing thymol or carvacrol	Edible coatings evaluated contained cassava starch, chitosan, and either LGRA106 (thymol at 59.26%) or LGRA107 (carvacrol at 43.24%)	YMC, *Pseudomonas aeruginosa*, *S. aureus*, *B. cereus*, *B. subtilis*, *Serratia*, *marcescens*, *E. coli*, *E. faecalis*, and *S. enteriditis*	Strawberries	The best formulation of edible coating (1.6% of cassava starch, 0.6% chitosan and 2.4% LGRA106 genotype) remained below the maximum limit recommended for total psychrophilic aerobic bacteria, YMC in strawberries during storage at 4 ºC for 7 days. Yeast and mould counts and total psychrophilic aerobic bacteria decreased from $1.7 \cdot 10^3$ to $6 \cdot 10^1$ CFU/g and from $1.5 \cdot 10^2$ to below 10 CFU/g, respectively.	(Azevedo et al., 2014)
Thiabendazole and cell-free supernatant obtained from *Bacillus subtilis* ET-1	The cell-free supernatant obtained from *B. subtilis*, Thiabendazole, and water were uniformly applied as a spray on the strawberry surface.	*B. cinerea* and *Penicillium digitatum*	Lemon and strawberry	Supernatant treatment of strawberry fruit reduced the incidence of disease from 96.4% to 22.3%. The percentage of surface area covered by gray mould was strongly reduced in treated strawberries when compared to the positive control. There were no disease incidences or decay signs in negative and chemical control.	(Ambrico & Trupo, 2017)

Table 2. Overview of physical technologies used alone or in combination with chemical sanitizers for the control of microorganisms on strawberries and products derived thereof.

Technology	Microorganisms evaluated	Food matrix	Conditions studied	Main outcomes	Reference
UV radiation	*E. coli* O157:H7 and *S. enterica*	Fresh strawberries	Intensity: 3-72 J/cm^2 Treatment time: 5-60 s**.**	Maximum reductions of *E. coli* O157:H7 and *Salmonella* were 3.9 and 3.4 log CFU/g, respectively and were achieved after 60 s and 72 J/cm^2.	(Bialka & Demirci, 2008)
UV radiation	TMC and YMC	Strawberry juice	Intensity: 254 nm, 25 ºC Treatment time: 15-60 min	Significant reduction by 2-log cycles in aerobic plate count as well as in total yeast and mould counts.	(Bhat, et al., 2015)
UV radiation	TMC and YMC	Strawberry nectar	Intensity: 230-2066 J/L at 8-10 ºC Flow rate: 4000 L/h Contact time: 0-12 min	Maximum reductions of TMC and YMC were 1.3- and 2.4-log CFU/mL. Authors suggested different doses for different products and therefore, the need for optimizing treatments depending on each product.	(Keyser, et al., 2008)
Combinations of UV radiation, IPL, and heat	Innoculated *B. cinerea*	Fresh strawberries	Intensity: 30 µs pulses, 15 Hz for IPL treatment; 0.5-1.0 kJ/m^2 for UV treatment, and 40-45 ºC for heat treatments. Treatment time: 40-250 s for IPL, 3-15 min for heat treatment	Short thermal treatments combined with IPL resulted in reduced fungal development. Combining two illumination treatments did not cause a significant decrease in fungal development. However, the most intense conditions increased the period before the first observation of fungal growth by 1 day.	(Marquenie, Michiels, Van Impe, Schrevens, & Nicolaï, 2003)
IPL	Postharvest disease assessed by incidence (visually recorded)	Fresh strawberries	Intensity: 2.4-47.8 J/cm^2 Treatment time: 2-40 s	The incidence of postharvest moulds on strawberry fruits was reduced by over 16-42% after IPL treatment.	(Duarte-Molina, et al., 2016)

WPL	*E. coli* O157:H7, *Salmonella*, and MNV-1	Fresh strawberries and raspberries	Intensity: 4.8-63.2 J/cm^2 combined with chemical sanitizers Treatment time: 5-60 s	*E. coli* inactivation was time-dependent. Processing for 60 s reduced *E. coli* O157:H7 from strawberries and raspberries by 2.4- and 4.5-log CFU/G, respectively. Combinations with chemical sanitizers resulted in higher efficacy in reducing *E. coli*. For decontamination of MNV-1, WPL processing for 60 s reduced the viral titers on strawberries and raspberries by 1.8- and 3.6-log units, respectively.	(Huang & Chen, 2015)
HPP	TMC and YMC	Strawberry puree	Intensity: 300 or 500 MPa (0 or 50 ºC). Treatment time: 1-15 min	HPP at 500 MPa at either 0 or 50 ºC reduced YMC from 4.6 and 3.8 log CFU/g to < 1 log CFU/g. HPP at 300 MPa allowed a reduction of 2.6 and 0.5 log CFU/g for YMC, respectively. HPP at 50 °C allowed a reduction of 4.7 log CFU/g in the TMC. No reductions were observed at 0 ºC.	(Marszałek, et al., 2015b)
HPP	*E. coli* O157:H7 and non-O157 STEC.	Strawberry puree	Intensity: 150-450 MPa Treatment time: 5-30 min	HPP at 350 MPa for more than 5 min allowed a reduction of 6-log CFU/g on non-O157 STEC.	(Hsu, et al., 2014)
HPP	Spores of *Byssochlamys nivea*	Strawberry puree	Intensity: 600 MPa Treatment time:	The 600 MPa HPP-thermal showed the best technique among HPTP, TS and thermal methods, for the inactivation of moulds' ascospores. For a 75 °C and 10 min HPTP process, 1.4 log reductions in ascospores of *B. nivea* were obtained. While after 40 min, reaching 3.4 log unit reductions for *B. nivea*. On the other hand, thermal treatment caused a steady and slow increase in the spore numbers. Although ≥12 min (*B. nivea*) TS processes showed higher inactivation (0.5 log) than thermal (no inactivation).	(Milani, Ramsey, & Silva, 2016)
HPP	Moulds, yeasts, *Alicyclobacillus*	Fruits including	Intensity: 600 MPa	*B. nivea* was more resistant to HPP combined with temperature than *N. fischeri*. For a 75 ºC and 10 min	(Milani & Silva, 2017)

	acidoterrestris, *B. nivea*, *Neosartorya fischery*, and spores of *Clostridium perfringenses* and *Bacillus cereus*	strawberries	Temperature: 70 or 75ºC Treatment time: 1-40 min	process, 1.4-log reductions in ascospores of *B. nivea* and 3.3-log reductions in ascospores of *N. fischeri* were obtained. HPP combined with temperature reduced the ascospores steadily, reaching 3.4-log for *B. nivea* and 5.2-log for *N. fischeri* after 40 min.	
HPP	MNV-1	Fresh strawberries and strawberry puree	Intensity: 350 MPa (0-20 ºC) Treatment time: 2 min	Pressure cycling offered no distinct advantage over continuous HPP. When operating in a dry state, lower temperatures resulted in increased inactivation of MNV-1. Treatment for 2 min at either 0 or 20 °C reduced the titer of MNV-1 by 4.4 and 0.5 log, respectively. In wet state, operating at 300 MPa and 0 °C achieved 2.9 log reductions of MNV-1.	(Huang, et al., 2014)
HPP	*E. coli* O157:H7 and *Salmonella* spp	Frozen strawberry puree	Intensity: 200-500 MPa (21 ºC) Treatment time: 2 min	HPP at 450 MPa for 2 min was able to eliminate both pathogens. Frozen storage at -18 ºC after HPP enhance the inactivation of both pathogens. Natural YMC were effectively reduced by HPP at 300 MPa for 2 min.	(Huang, et al., 2013)
HPP	MNV-1	Strawberry puree and water	Intensity: 200-600 MPa Treatment time: 2.5-10.0 min	The reduction in MNV-1 infectivity achieved was pressure- and matrix-dependent. HPP at 400 MPa for 2.5 min proved to be sufficient for inactivation of MNV-1 with over 99.9% reduction.	(Mosqueda-Melgar, et al., 2012)
PEFs	*E. coli* O157:H7 and *Salmonella* Enteritidis	Fruit juices including juice	Intensity: 35 kV/cm combined with chemical sanitizers Treatment time: 500-2000 µs	S. Enteritidis and *E. coli* O157:H7 were reduced by more than 5-log units in orange juice treated by PEFs; whereas strawberry, apple, and pear juices were pasteurized when the PEFs were combined with chemical sanitizers.	(Mosqueda-Melgar, et al., 2008)
PEFs	*E. coli* and *E. coli* O157:H7	Strawberry juice	Intensity: 18.6 kV/cm combined with antimicrobials (45-55 ºC)	Inactivation of *E. coli* at 45, 50, and 55°C were 2.86, 3.12, and 3.79 log CFU/mL. Inactivation of *E. coli* O157:H7 under the same conditions were 3.09, 4.08, and	(Gurtler, et al., 2011)

			Treatment time: 150 µs	4.71 log CFU/mL, respectively. Combinations with chemical treatments enhanced the efficacy of the process.	
CAP	*S. enterica* serovar *Typhimurium*	Inoculated fresh produce including strawberries	Nitrogen-CAP at <35ºC for 1-15 min	Maximum reductions were obtained after 15 min of treatment and were 2.7-, 1.7-, and 0.9-log for *Salmonella* inoculated on lettuce, strawberry, and potato, respectively.	(Fernandez, Noriega, & Thompson, 2013)
CAP	Aerobic mesophillic bacteria and yeast and mould count	Inoculated strawberries	CAP at 25 ºC during 5 min	Treatment for 5 min resulted in 2.4- and 3.3-log reductions in the total mesophilic and YMC, respectively. Ozone was generated inside the package and approximately 1000 ppm were measured immediately post-treatment.	(Misra, et al., 2014a)
CAP	TMC, YMC, *E. coli*, *S. enterica* serovar *Typhimurium*, and *L. monoxytogenes*	Inoculated strawberries	Ozone CAP for 10-120 s	Reductions in the TMC and YMC were calculated after 60 s treatments as 1.6- and 5.5-log CFU/g, respectively. Treatment for 120 s significantly reduced *L. monocytogenes* inoculated on strawberries. Higher processing times did not yield any further reductions of bacteria.	(Ziuzina, et al., 2014)
Plasma-activated water	*Staphylococcus aureus*	Inoculated strawberries	Plasma-activated water with continuous agitation for 5-15 min	Plasma-activated water treatments achieved initial reductions of *S. aureus* ranging from 1.6- to 2.3-log. These reductions ranged between 1.7- to 3.4-log after 4 days of storage. After the storage at 20 °C during 6 days, no visual fungal spoilage was detected on treated strawberries.	(Ma, Wang, Tian, Wang, Zhang, & Fang, 2015)
Ionizing radiation	NoV and Tulane virus	Fresh strawberries	E-beam: : 4-28 kGy Gamma irradiation: 2.8-	A high dose of E-beam treatment was required to completely abolish the receptor binding ability of human	(DiCaprio, et al., 2016)

			22.4 kGy	NoV (35.3 kGy) and Tulane virus (19.5–24.1 kGy). Both human NoV and TV were more susceptible to gamma irradiation than E-beam.	
Thermosonication	Moulds, yeasts, *Alicyclobacillus acidoterrestris*, *S. nivea*, *N. fischery*, and spores of *Clostridium perfringenses* and *B. cereus*	Strawberry puree	Intensity: 24 kHz Temperature < 78ºC	Thermosonication showed higher inactivation (0.5-log) than thermal (no inactivation). An unexpected increase in the spore number up to a maximum of 1.0-log for *B. nivea* (at 5 min) and 2.4-log for *N. fisheri* (at 10 min), prior to inactivation, makes the 0.33 W/mL 75 ºC thermosonication process not feasible for commercial application.	(Milani, et al., 2017)
US	TBC and yeast and mould count	Fresh strawberries	Intensity: 20 kHz, 30-90 W Treatment time: 5-10 min	No differences found between US treatments. US processing reduced the percentage of infected strawberries after 1 week of storage at 4 °C from 6% (control) to 0%, and after 4 weeks of storage from 17% (control) to 6%.	(Aday, Büyükcan, & Caner, 2013)
US	TBC and yeast and mould count	Fresh strawberries	Intensity: 33 kHz, 60 W Treatment time: 0-60 min	At the initial day, the bacterial count decreased from 3.60 to 2.1- and 2.0-log CFU/g and yeast and mould count decreased from 3.5- to 2.2- and 2.0-log CFU/g, after 40 and 60 min of treatment time, respectively. After storage of samples at 4 °C for 15 days, the bacteria load increased to 5.9-, 3.9-, and 5.3-log CFU/g, when samples were processed for 0, 40, or 60 min, respectively. Similar results were observed in yeast and mould, reaching populations of 4.8-, 3.5-, and 4.3-log CFU/g, at 0, 40, or 60 min treatment time, respectively.	(Gani, et al., 2016)
US	Aerobic mesophiles bacteria and YMC	Fresh watercress, parsley, and strawberries	Intensity: 45 kHz in combination with chemical sanitizers Treatment time: 10 min	US combination with sanitizers increased their efficiency. All evaluated treatments of strawberry reduced aerobic mesophiles from 0.7- to 4.0-log cycles. The combined treatment with US and 40 mg/L PAA resulted in the highest reduction in the natural contaminant population.	(São José, et al., 2015)

US	Moulds	Fresh strawberries	Intensity: 20 kHz, 30 W combined with chemical sanitizers Treatment time: 5 min	All treatments prevented mould growth when compared to the control. After storage at 4 °C, untreated fruit had 21% and 35% decay during the third and fourth weeks, respectively.	(Aday, & Caner, 2014)
US	YMC, mesophilic aerobic, lactic acid bacteria, and inoculated *S. enterica*	Fresh strawberries	Intensity: 40 kHz, 500 W combined with chemical sanitizers Treatment time: 5 min	US increased the effect of all chemical compounds in the reduction of aerobic and mesophilic bacteria and YMC. US combined with PAA reduced 1.8-, 2.0-, and 2.0-log CFU of YMC, mesophilic aerobic bacteria, and lactic acid bacteria, respectively. US processing reduced *S. enterica* population almost 0.6-log units.	(do Rosário, et al., 2017)
US	*E. coli* O157:H7	Inoculated strawberries	Intensity: 44-48 kHz combined with chemical sanitizers Treatment time: 5 min	US combined with chlorinated water or Acidic EOW reduced *E. coli* O157:H7 cells by 0.7- to 1.9-log CFU/g depending on the treatment time and treatment solution temperature.	(Hung, et al., 2010)
US	TAB and YMC	Fresh fruits including strawberries	Intensity: 40 kHz, 240 W combined with acidic EOW Treatment time: 10 min	US enhanced the bactericidal activity of acidic EOW which resulted in 1.7- and 1.2-log reductions on TAB, and 1.5- and 1.2-log reductions on YMC, respectively for cherry tomatoes and strawberries.	(Ding, et al., 2015)
US combined with chemicals	Natural contaminant population	Watercress, parsley and strawberries	Intensity: 45 kHz, 10 min. Combined with: 20 & 200 mg/L sodium dichloroisocyanurate, 5% hidrogen peroxide, 10 mg/L chlorine dioxide or 400 mg/l PAA	The reductions of aerobic mesophiles in strawberries ranged between 0.7- and 4.0-log units, being the combination with PAA the most effective. However, all treatments with US promoted a reduction in strawberry firmness	(de Sao José & Vanetti, 2015)

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