Chitosan, a Biopolymer With Triple Action on Postharvest Decay of Fruit and Vegetables: Eliciting, Antimicrobial and Film-Forming Properties

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Chitosan is a natural biopolymer from crab shells that is known for its biocompatibility, biodegradability, and bioactivity. In human medicine, chitosan is used as a stabilizer for active ingredients in tablets, and is popular in slimming diets. Due to its low toxicity, it was the first basic substance approved by the European Union for plant protection (Reg. EU 2014/563), for both organic agriculture and integrated pest management. When applied to plants, chitosan shows triple activity: (i) elicitation of host defenses; (ii) antimicrobial activity; and (iii) film formation on the treated surface. The eliciting activity of chitosan has been studied since the 1990’s, which started with monitoring of enzyme activities linked to defense mechanisms (e.g., chitinase, β-1,3 glucanase, phenylalanine ammonia-lyase) in different fruit (e.g., strawberry, other berries, citrus fruit, table grapes). This continued with investigations with qRT-PCR (Quantitative Real-Time Polymerase Chain Reaction), and more recently, with RNA-Seq. The antimicrobial activity of chitosan against a wide range of plant pathogens has been confirmed through many in-vitro and in-vivo studies. Once applied to a plant surface (e.g., dipping, spraying), chitosan forms an edible coating, the properties of which (e.g., thickness, viscosity, gas and water permeability) depend on the acid in which it is dissolved. Based on data in literature, we propose that overall, the eliciting represents 30 to 40% of the chitosan activity, its antimicrobial activity 35 to 45%, and its film-forming activity 20 to 30%, in terms of its effectiveness in the control of postharvest decay of fresh fruit. As well as being used alone, chitosan can be applied together with many other alternatives to synthetic fungicides, to boost its eliciting, antimicrobial and film-forming properties, with additive, and at times synergistic, interactions. Several commercial chitosan formulations are available as biopesticides, with their effectiveness due to the integrated combination of these three mechanisms of action of chitosan.

Keywords: antimicrobial activity, biopolymer, coating, induced resistance, natural fungicide
INTRODUCTION

Chitosan is the linear polysaccharide of glucosamine and N-acetylglucosamine units joined by β-1,4-glycosidic links and it is obtained by deacetilation of chitin through exposure to NaOH solutions or to the enzyme chitinase. Chitosan and chitin are naturally occurring polymers. For their biocompatibility and biosafety, their applications are widespread in many industries, such as cosmetology, food, biotechnology, pharmacology, medicine, and agriculture (Ding et al., 2013; Lei et al., 2014). In particular, chitosan has increasing interest in plant protection as a natural fungicide and plant defense booster, and meets the interest of many researchers, that used it to prolong the storage of an array of fruit and vegetables worldwide. Chitosan was the first compound in the list of basic substances approved in the European Union for plant protection purposes (Reg. EU 662014/563), for both organic agriculture and integrated pest management. A comprehensive review on the available data on the effectiveness of chitosan was published recently, for its preservation of fruit and vegetables, both alone and in combination with other treatments, and its mechanisms of action (Romanazzi et al., 2017). However, the increasing knowledge of this biopolymer (Figure 1) and the fast advances in basic and applied research in this field require a more focused and schematic update based on the last 5 years of investigations (2013–2018). The reader can then focus on specific aspects from the long list of other reviews that have appeared on the subject, among which some have focused on the applications of chitosan to fruit and vegetables (Bautista-Banos et al., 2006; Bautista-Baños et al., 2016; Zhang et al., 2011). When applied to plants, chitosan shows triple activity: (i) elicitation of host defenses; (ii) antimicrobial activity; and (iii) film formation on the treated surface. We will cover the recent information on these issues in the following sections, which is also listed comprehensively in the Tables, with examples of these applications.

EFFECTIVENESS OF CHITOSAN IN THE CONTROL OF POSTHARVEST DECAY OF FRUIT

The potential effectiveness of chitosan as a coating for fresh fruit was first proposed by Muzzarelli (1986). The first in-vivo application of chitosan on fruit was in the Josep Arul Laboratory, by Ahmed El Ghaouth, who produced a list of papers through the last decade of the last century. These included El Ghaouth et al. (1992), where they applied chitosan to strawberries and other fruit, both alone and in combinations with other potential biocontrol agents, which then contributed to the development of some commercial formulations. Following these promising investigations, and with the growing need for alternatives to the use of synthetic fungicides, chitosan use became popular, and it was proposed to be part of a new class of plant protectants (Bautista-Baños et al., 2006). Chitosan coatings have now been applied to numerous temperate and subtropical fruit, both alone and in combination with other treatments (Tables 1–3), with generally additive, and in some cases synergistic, effectiveness (Romanazzi et al., 2012).

CHITOSAN ELICITING ACTIVITY

Chitosan is known to elicit plant defences against several classes of pathogens, including fungi, viruses, bacteria and phytoplasma (El Hadrami et al., 2010). Moreover, in some studies, its eliciting activity was reported to be effective on pests (Badawy and Rabea, 2016). Based on our experience, the eliciting activity of chitosan accounts for 30 to 40% of its effectiveness in the control of postharvest decay of fresh fruit (Figure 2). The extent of this eliciting activity depends on the reactivity of the fruit tissues, and it is well known that fruit responses to stress decline with ripening (Romanazzi et al., 2016). This eliciting activity of chitosan has been studied since the 1990’s, which started with monitoring of
| Fruit          | Decay agent                                      | Combination with chitosan                                                                 | Reference                          |
|---------------|------------------------------------------------|-----------------------------------------------------------------------------------------|-----------------------------------|
| Table grapes  | Botrytis cinerea                                | Salicylic acid                                                                          | Shen and Yang, 2017               |
|               | General decay                                   | Glucose complex                                                                         | Gao et al., 2013                  |
|               | Aspergillus niger, Rhizopus stolonifer          | –                                                                                        | de Oliveira et al., 2014          |
|               | Fusarium oxysporum                              | –                                                                                        | Irkin and Guldas, 2014            |
|               | General decay                                   | –                                                                                        | Feliziani et al., 2013a           |
|               | General decay                                   | Ultraviolet-C                                                                          | Freitas et al., 2015              |
|               | General decay                                   | –                                                                                        | Al-Quraishi and Mohamed, 2015     |
|               | Aspergillus niger, Botrytis cinerea, Penicillium expansum, Rhizopus stolonifer | Menta essential oil                                                                     | Guerra et al., 2016               |
|               | Botrytis cinerea                                | Salvia officinals essential oil                                                         | Kanetis et al., 2017              |
| Strawberry    | Botrytis cinerea                                | Lavender and thyme essential oil                                                        | Sangsuwan et al., 2016            |
|               | General decay                                   | Poeny extract                                                                           | Pagliarulo et al., 2016           |
|               | Penicillium expansum, Rhizopus stolonifer       | Olive oil processing waste                                                              | Khalifa et al., 2016              |
|               | Total microbial load                            | Natamycin, nisin, pomegranate, grape seed extract                                        | Duran et al., 2016                |
|               | Total microbial load                            | Quinoa protein-chitosan and quinoa protein-chitosan-sunflower oil                        | Valenzuela et al., 2015           |
|               | Botrytis cinerea                                | Sodium benzoate and potassium sorbate                                                   | Treviño-Garza et al., 2015        |
|               | Rhizopus stolonifer                             | Zataria multiflora essential oil                                                        | Mohammadi et al., 2015            |
|               | General decay                                   | Cinnamon leaf essential oil containing oleic acid                                       | Perdones et al., 2014             |
|               | General decay                                   | Geraniol and thymol                                                                     | Benhabiles et al., 2013           |
|               | General decay                                   | Carboxymethyl cellulose, hydroxypropylmethyl cellulose                                  | Gol et al., 2013                  |
|               | Botrytis cinerea                                | Nanosized silver-chitosan composite                                                     | Moussa et al., 2013               |
|               | General decay                                   | Beeswax                                                                                 | Velickova et al., 2013            |
|               | Botryosphaeria sp.                              | –                                                                                        | Wang et al., 2017                 |
| Pear          | General decay                                   | Cellulose nanocrystals                                                                  | Deng et al., 2017                 |
|               | General decay                                   | Acylated soy protein isolate and stearic acid                                          | Wu et al., 2017                   |
| Apple         | General decay                                   | Olive waste extracts                                                                   | Khalifa et al., 2015, 2016        |
|               | Penicillium expansum                            | –                                                                                        | Darolt et al., 2016               |
|               | Venturia inaequalis                             | –                                                                                        | Felipini et al., 2016             |
|               | Penicillium expansum                            | –                                                                                        | Li et al., 2015                   |
|               | Calyx senescence                                | V                                                                                       | Deng et al., 2016                 |
| Citrus        | Penicillium digitatum, Penicillium italicum     | Silver nanoparticles                                                                    | Al-Sheikh and Yehia, 2016         |
|               | Colletotrichum gloeosporioides                  | *Pichia membranaefaciens*                                                               | Zhou et al., 2016                 |
|               | Penicillium digitatum, Penicillium italicum     | Cress and/or pomegranate extracts                                                       | Tayel et al., 2016                |
|               | Penicillium digitatum                           | Clove oil                                                                               | Shao et al., 2015                 |
|               | Penicillium digitatum                           | Cyclic lipopeptide antibiotics from *Bacillus subtilis*                                  | Waewthongrak et al., 2015         |
|               | General decay                                   | Carboxymethyl cellulose                                                                 | Arnon et al., 2014                |
|               | Total microbial load                            | Carboxymethyl cellulose and sealed with chitosan/boehmite nanocomposite lidding films   | Kaur et al., 2017                 |
| Peach         | Monilinia laxa                                  | Silver nanoparticles                                                                    | Ma et al., 2013                   |
|               | General decay                                   | Polyethylene terephthalate punnets containing thyme oil and sealed with chitosan/boehmite nanocomposite lidding films | Fabrizi et al., 2013b            |
|               | Total microbial load                            | Carboxymethyl cellulose                                                                 | Cindi et al., 2015                |
|               | Sweet cherry                                    | Polyethylene terephthalate punnets containing thyme oil and sealed with chitosan/boehmite nanocomposite lidding films | Pasquariello et al., 2015       |
|               | General decay                                   | γ-ray                                                                                    | Elbarbary and Mostafa, 2014       |
|               | Monilinia fructicola                            | –                                                                                        | Ma et al., 2013                   |
|               | Monilinia laxa, Botrytis cinerea, Rhizopus stolonifer | –                                                                                      | Feliziani et al., 2013b           |
| Plum          | General decay                                   | Hydroxypropyl methylcellulose                                                          | Sharmugha Priya et al., 2014      |
|               | General decay                                   | Ascorbic acid                                                                           | Liu et al., 2014                  |
TABLE 2 | Postharvest chitosan treatments with other applications for storage decay of subtropical fruit.

| Fruit     | Decay agent                                      | Combination with chitosan                                  | Reference                          |
|-----------|-------------------------------------------------|-----------------------------------------------------------|-----------------------------------|
| Mango     | Anthracnose (Colletotrichum gloeosporioides)     | Spermidine                                                | Jongsri et al., 2017              |
|           | Anthracnose (Colletotrichum gloeosporioides),   | Lactoperoxidase system incorporated chitosan films         | Kouakou et al., 2013              |
|           | stem-end rot (L. theobromae strains)            |                                                           |                                   |
| Anthracnose|                                                 | Mentha piperita L. essential oil                           | de Oliveira et al., 2017          |
| Anthracnose| (Colletotrichum gloeosporioides),                | Lactoperoxidase system incorporated chitosan films         | Kouakou et al., 2013              |
|           | stem-end rot (L. theobromae strains)            |                                                           |                                   |
| Anthracnose|                                                 | Mentha piperita L. essential oil                           | de Oliveira et al., 2017          |
| Citrus    | Green mold (Penicillium digitatum)              | Bacillus subtilis ABS-S14                                  | Waewthongrak et al., 2015         |
| Avocado   | Anthracnose (Colletotrichum gloeosporioides)     | Thyme oil                                                  | Bill et al., 2014                 |
| Tomato    | Alternaria alternata                             | Methyl jasmonate                                           | Chen et al., 2014                 |
| Pomegranate| Penicillium spp., Pilidiella granati             | Essential oil from Origanum vulgare L. Lemongrass film     | Barreto et al., 2016              |

TABLE 3 | Preharvest chitosan treatments with other applications for storage decay of temperate fruit.

| Fruit     | Decay            | Combination with chitosan                                  | Reference                          |
|-----------|------------------|-----------------------------------------------------------|-----------------------------------|
| Citrus    | Penicillium digitatum | Rhodosporidium paludigenum                                   | Lu et al., 2014                    |
| Peach     | General decay    | Calcium chloride                                           | Gayed et al., 2017                |
| Jujube fruit | Alternaria alternata | –                                                       |                                   |
| Table grapes | Botrytis cinerea | Salicylic acid                                              | Shen and Yang, 2017                |
| Strawberry | Botrytis cinerea and Rhizopus stolonifer         | –                                                       | Romanazzi et al., 2013; Feliziani et al., 2015 |
| Strawberry | Botrytis cinerea | –                                                       | Lopes et al., 2014                 |
| Strawberry | General decay    | –                                                       | Saavedra et al., 2016              |
| Sweet cherry | Monilinia laxa, Botrytis cinerea, and Rhizopus stolonifer | –                           | Feliziani et al., 2013a            |

FIGURE 2 | Proportion of antimicrobial, eliciting, and film-forming properties of chitosan.

the activities of enzymes linked to the defense mechanisms (e.g., chitinase) in different fruit (e.g., strawberries) (El Ghaouth et al., 1992). This was followed by investigations on other berries, citrus fruit and table grapes, among others. More recently, tools such as qRT-PCR and in recent years RNA-Seq (RNA-Sequencing) have allowed important information to be gained, first at the level of single gene expression, and then later at the level of global gene expression (Xoca-Orozco et al., 2017). This has provided good understanding of the multiple actions of chitosan applications and how they affect a number of physiological changes in fruit. As an example, the application of chitosan to strawberries at different times before harvest can affect the expression of a thousand or more genes (Landi et al., 2017). Some examples that have become available in the literature over the last 5 years are listed in Table 4, which deal with the physiological changes that can occur in chitosan-treated fruit, both when the biopolymer is applied alone, and when it is combined with other treatments. The eliciting activity of chitosan is particularly effective toward latent infections, as a more reactive fruit can stop the infection process, through a balance that resembles quorum sensing, which is well known for bacterial infections (Papenfort and Bassler, 2016).

CHITOSAN ANTIMICROBIAL ACTIVITY

Numerous studies on chitosan inhibitory activities toward numerous microorganisms have been carried out since the first report of almost half a century ago (Allan and Hadwiger, 1979). The antimicrobial activities of chitosan against a wide range of plant pathogens have been confirmed by any of in-vitro and in-vivo studies. The antimicrobial activity of chitosan is one of its main properties, and this depends on the concentration at which it is applied. In the control of postharvest decay of fresh fruit, the antimicrobial activity can account for 35–45% of its effectiveness, as an antifungal barrier on a fruit inhibits the germination of fungal spores and slows down the rate of decay-causing fungi of already infected fruit, both latently and
### TABLE 4 | Physiological changes that can occur in fresh fruit after chitosan treatment, alone or in combination with other applications.

| Fruit       | Physiological change                                                                                                                                  | Combination with chitosan                                                                 | Reference                        |
|-------------|------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|----------------------------------|
| Apple       | 20 genes involved in defence responses, metabolism, signal transduction, transcription factors, protein biosynthesis, cytoskeleton. Total phenolic, flavonoids, antioxidants, pigments, weight loss | Olive waste extract                                                                      | Li et al., 2015                  |
|             | Malondialdehyde content                                                                                                                                  | γ-ray                                                                                     | Elbarbary and Mostafa, 2014      |
|             | Catalase, peroxidase, β-1,3-glucanase and chitinase                                                                                                      | –                                                                                        | Ma et al., 2013                   |
|             | Total soluble solids, weight loss, ascorbic acid content                                                                                                 | Silver and zinc oxide nanoparticles                                                      | Kaur et al., 2017                |
|             | Color and fruit firmness                                                                                                                                  | Polyethylene terephthalate punnets containing thyme oil and sealed with chitosan/boehmite nanocomposite lidding films | Cindi et al., 2015               |
|             | Fruit firmness, weight loss, total soluble solids, total phenolic content, and titratable acidity                                                      | Calcium chloride                                                                         | Gayed et al., 2017               |
| Plum        | Fruit firmness, respiration rate, fruit color, polygalacturonase, superoxide dismutase, catalase, polyphenol oxidase, phenylalanine ammonia lyase and pectin methyl esterase activities, superoxide free radicals, malondialdehyde content | Ascorbic acid                                                                            | Liu et al., 2014                  |
| Sweet cherry| Malondialdehyde content and superoxide dismutase, catalase, ascorbate peroxidase, polyphenol oxidase, guaiacol peroxidase lipoygenase activities | –                                                                                        | Pasquariello et al., 2015         |
| Strawberry  | Over 5000 differently expressed genes                                                                                                                 | –                                                                                        | Landi et al., 2017                |
|             | 18 defence genes                                                                                                                                                                                                 | –                                                                                        | Landi et al., 2014                |
|             | Fruit color                                                                                                                                              | –                                                                                        | Feliziani et al., 2015            |
|             | Fruit firmness, anthocyanin and total phenol content                                                                                                                                                             | Carboxymethyl cellulose, hydroxypropylmethyl cellulose                                   | Gol et al., 2013                  |
|             | Weight loss, titratable acidity, pH, total soluble solids, total phenols, anthocyanin and ascorbic acid content, activity of polygalacturonase, pectin methyl esterase, β-galactosidase and cellulose | Calcium chloride                                                                         | Gayed et al., 2017               |
|             | Weight loss                                                                                                                                              | –                                                                                        | Natamycin, nisin, pomegranate, grape seed extract                                        | Duran et al., 2016                |
|             | Titratable acidity, soluble solids content                                                                                                                                                                       | Poey extract                                                                             | Pagliarulo et al., 2016           |
|             | pH and soluble solids content                                                                                                                                                                                      | Beeswax                                                                                 | Velickova et al., 2013            |
|             | Weight loss, ascorbic acid                                                                                                                                                                                          | –                                                                                        | Iglesias et al., 2013             |
|             | Weight loss, respiration rate, skin and flesh color, firmness, pH, titratable acidity, soluble solids content, reducing sugars content                                                        | Sodium benzoate, potassium sorbate                                                         | Treviso-Garza et al., 2015         |
|             | Weight loss, firmness, color and total soluble solids content                                                                                                                                                     | Carboxymethyl cellulose, hydroxypropylmethyl cellulose                                   | Gol et al., 2013                  |
|             | Weight losses, total soluble solids and titratable acidity                                                                                                                                                        | Olive waste extract                                                                      | Khalifa et al., 2016              |
|             | Allergen-related gene                                                                                                                                                                                              | –                                                                                        | Petriccione et al., 2017          |
| Table grapes| Phenylalanine ammonia lyase, chitin, and β-1, 3-glucanase, phenolic compounds, respiration rate, weight loss, total soluble solids, titratable acidity                                                                 | Salicylic acid                                                                           | Shen and Yang, 2017               |
|             | Total phenols, flavonoids and ascorbic acid content, activities of peroxidase, polyphenoloxidase, polygalacturonase, and xylanase, fruit firmness                                                                    | –                                                                                        | Al-Qurashi and Mohamed, 2015       |
|             | Fruit color                                                                                                                                              | Ultrasound                                                                               | Freitas et al., 2015              |
|             | Weight loss, titratable acidity, pH and soluble solids content, resveratrol content                                                                                                                            | Salvia officinalis essential oil                                                         | Kanetis et al., 2017              |
|             | Weight loss, soluble solids content and titratable acidity                                                                                                                                                        | Menta essential oil                                                                     | Guerra et al., 2016               |
|             | Firmness, titratable acidity, soluble solids, color, weight loss                                                                                                                                                  | Glucose complex                                                                          | Gao et al., 2013                  |
|             | Total soluble solids, ascorbic acid content, titratable acidity, weight loss, respiration rate, activities of peroxidase and superoxide dismutase                                                               | Carboxymethyl cellulose                                                                  | de Oliveira et al., 2014          |
|             | Titratable acidity, soluble solids, color, firmness                                                                                                                                                              | Cyclic lipopeptide antibiotics from Bacillus subtilis                                    | Amon et al., 2014                 |
| Citrus      | Chitinase activity, quercetin, myricetin, and resveratrol content                                                                                                                                                 | –                                                                                        | Feliziani et al., 2013b           |
|             | Chitinase and phenylalanine ammonia lyase                                                                                                                                                                           | –                                                                                        | Lu et al., 2014                   |
|             | 640 differently expressed genes, many involved in secondary metabolism and hormone metabolism pathways                                                                                                        | –                                                                                        | Coqueiro et al., 2015             |
|             | Fruit firmness, weight loss, total soluble solids                                                                                                                                                                 | Carboxymethyl cellulose                                                                  | Amon et al., 2014                 |
|             | Peroxidase and phenylalanine ammonia-lyase                                                                                                                                                                         | Cyclic lipopeptide antibiotics from Bacillus subtilis                                    | Waewthongrak et al., 2015         |
|             | Contents of chlorophylls and total carotenoids                                                                                                                                                                    | –                                                                                        |                                 |
|             | Phenylalanine ammonia-lyase, β-1,3-glucanase, chitinase                                                                                                                                                            | –                                                                                        |                                 |

(Continued)
TABLE 4 | Continued

| Fruit | Physiological change | Combination with chitosan | Reference |
|-------|---------------------|--------------------------|-----------|
| Jujube | Fruit firmness, cellulase, pectinase | – | Guo et al., 2017 |
| Pear  | Total phenolic and flavonoid contents, superoxide dismutase, peroxidase and catalase activities, total antioxidant activity | Calcium chloride | Kou et al., 2014a |
| Mango | Malic acid-metabolising enzymes and related genes expression | Calcium chloride | Kou et al., 2014b |
| Kiwifruit | Peroxidase (POD) and polyphenol oxidase (PPO) gene expression | – | Gutiérrez-Martínez et al., 2017 |
|       | Induced gene expression and increased enzymatic activity of catalase, superoxide dismutase and ascorbate peroxidase | – | Zheng et al., 2017 |

TABLE 5 | Some chitosan-based commercial products that are available for control of postharvest diseases of fruit and vegetables.

| Product trade name | Company (Country) | Formulation | Active ingredient (%) |
|--------------------|-------------------|-------------|-----------------------|
| Chito plant        | ChiPro GmbH (Bremen, Germany) | Powder | 99.9 |
| Chito plant        | ChiPro GmbH (Bremen, Germany) | Liquid | 2.5 |
| Oii-YS             | Venture Innovations (Lafayette, LA, United States) | Liquid | 5.8 |
| KaltoSol           | Advanced Green Nanotechnologies Sdn Bhd (Cambridge, United Kingdom) | Liquid | 12.5 |
| Armour-Zen         | Botry-Zen Limited (Dunedin, New Zealand) | Liquid | 14.4 |
| Biorend            | Bioagro S.A. (Chile) | Liquid | 1.25 |
| Kforce             | Alba Milagro (Milan, Italy) | Liquid | 6 |
| FreshSeal          | BASF Corporation (Mount Olive, NJ, United States) | Liquid | 2.5 |
| ChitoClear         | Primex ehf (Siglufjordur, Iceland) | Powder | 100 |
| Bioshield          | Seafresh (Bangkok, Thailand) | Powder | 100 |
| Biochikol 020 PC   | Gumitex (Lovich, Poland) | Liquid | 2 |
| Kadozan            | Lytone Enterprise, Inc. (Shanghai Branch, China) | Liquid | 2 |
| Kendal cops        | Valagro (Atessa, Italy) | Liquid | 4 |
| Chitosan 87%       | Korea Chengcheng Chemical Company (China) | TC (Technical material) | 87 |
| Chitosan 2%        | Korea Chengcheng Chemical Company (China) | SLX (Soluble concentrate) | 2 |

actively (Figure 2). A standard application rate of chitosan to provide a significant control of postharvest decay of fruit and vegetables can be considered 1%, except for the control of Penicillia, where higher concentrations may be needed to provide a good effectiveness. The degree of deacetylation and the molecular weight of chitosan characterize its properties, such as the number of positively charges of amino groups and therefore, its electrostatic interactions with different substrate and organisms at different pH. Chitosan with a higher degree of deacetylation, which has greater numbers of positive charges, would also be expected to have stronger antibacterial activities. On the other hand, numerous studies have generated different results relating to correlations between the chitosan bactericidal activities and its molecular weight (Romanazzi et al., 2017). In addition, there are many differences between the chitosan antifungal and antibacterial activities and several mechanisms relating to these remain still unclear and further researches are needed (Romanazzi et al., 2017).

CHITOSAN FILM-FORMING PROPERTIES

Once applied to a plant surface by dipping or spraying, chitosan can form an edible coating, the properties of which (e.g., thickness, viscosity, gas, and water permeability) greatly depend on the acid in which the biopolymer is dissolved. The film-forming properties of chitosan account for 20–30% of the chitosan effectiveness in the control of postharvest decay of fruit and vegetables (Figure 2). Coating produces a barrier for gas exchanges and reduced respiration, and slows down fruit ripening. Of note, a less ripe fruit is less sensitive to postharvest decay.

TOWARD LARGE-SCALE COMMERCIAL APPLICATIONS

When first used in experimental trials, chitosan needed to be dissolved in an acid (e.g., hydrochloric acid, acetic acid, which were among the most effective ones; see Romanazzi et al., 2009), and then taken to the optimal pH (∼5.6) This approach can even take 1–2 days, and it is impractical for use by growers. More recently, several commercial chitosan formulations that can be dissolved in water have become available on the market to be used as a biopesticides (Table 5). Some of these are formulated as powders, and then the cost of shipping is lower (although still higher compared to most of the commercially available synthetic fungicides), although the chitosan needs to be dissolved in water, in some cases a few hours before its application. This makes chitosan more difficult to use, as the grower wants to use an alternative to synthetic fungicides in
CONCLUDING REMARKS

The effectiveness of chitosan application arises from the integrated combination of its three mechanisms of action. There are increasing consumer requests for fruit and vegetables to be free from residues of synthetic pesticides, such that the rules defined by the public administration have become more limiting in terms of the active ingredients allowed and the maximum residue limits. Also, large stores compete with each other to further reduce these limits, compared to the legal thresholds (Romanazzi et al., 2016b). These trends make the concept of the application of alternatives to synthetic fungicides more popular, and among these the main one that is already used in human medicine is chitosan, which is particularly welcomed by public opinion. These aspects have promoted further studies based on the multiple actions of chitosan on fruit and vegetables. Therefore, further increases in our knowledge are expected following the widespread practical application of chitosan due to the regulation of its use in agriculture and the interest of companies to promote chitosan-based products, with potential benefits for the growers, the consumers and the environment.

AUTHOR CONTRIBUTIONS

GR proposed the review, collected data on chitosan popularity over time and on commercial products, coordinated the authors, and wrote the article. EF collected papers on effectiveness of chitosan on temperate fruit and on the mechanisms of action in the tables, and helped with the writing. DS collected papers on effectiveness of chitosan on tropical fruit and on the mechanisms of action in the tables, and helped with the writing.
development in mango cultivar Tommy Atkins. *Food Microbiol.* 66, 96–103. doi: 10.1016/j.fm.2017.04.012

Deng, L., Yin, B., Yao, S., Wang, W., and Zeng, K. (2016). Postharvest application of oligochitosan and chitosan reduces calyx alterations of citrus fruit induced by ethylene degreasing treatment. *J. Agric. Food Chem.* 64, 7394–7403. doi: 10.1021/acs.jafc.6b01217

Deng, Z., Jung, J., Simonsen, J., Wang, Y., and Zhao, Y. (2017). Cellulose nanocrystal reinforced chitosan coatings for improving the storability of postharvest pears under both ambient and cold storages. *J. Food Sci.* 82, 453–462. doi: 10.1111/1750-3841.13601

Ding, F., Nie, Z., Deng, H., Xiao, L., Du, Y., and Shi, X. (2013). Antibacterial hydrogel coating by electrophoretic co-deposition of chitosan/alkynyl chitosan. *Carbohydr. Polym.* 98, 1547–1552. doi: 10.1016/j.carbpol.2013.07.042

Duran, M., Aday, M. S., Zorba, N. N. D., Temizkan, R., Büyükcan, M. B., Feliziani, E., Santini, M., Landi, L., and Romanazzi, G. (2013b). Pre- and postharvest application of submicron chitosan dispersions for controlling *Alternaria* rot in postharvest jujube fruit. *Postharv. Biol. Technol.* 84, 112–121. doi: 10.1016/j.postharvbio.2012.12.004

Felipini, R. B., Boneti, J. I., Katsurayama, Y., Neto, A. C. R., Veleirinho, B., Feliziani, E., Santini, M., Landi, L., and Romanazzi, G. (2013a). Preharvest fungicide, potassium sorbate, or chitosan application of differentially expressed genes in strawberry after preharvest application of calcium chloride and chitosan coating treatment on quality attributes and storage behavior of harvested litchi fruit. *Food Chem.* 252, 134–141. doi: 10.1016/j.foodchem.2018.01.095

Guo, S., et al. (2013a). Preharvest fungicide, potassium sorbate, or chitosan by lactoperoxidase system. *Food Hydrocoll.* 74, 739–744. doi: 10.1016/j.foodhyd.2013.02.011

Guo, H., Xing, Z., Yu, Q., Zhao, Y., and Zhu, E. (2017). Effectiveness of preharvest application of calcium chloride and chitosan on control of postharvest blue mold decay of apple fruit (*Malus domestica* var. Anna) fruit bioactive substances using olive wastes extract-chitosan film coating. *Innov. Food Agric.* 4, 90–99. doi: 10.1002/Infa.2016.11.001

Guo, X. H., Guo, W. L., Guo, R. Z., Li, X. Y., and Xue, Z. H. (2014a). Effects of chitosan, calcium chloride, and pullulan coating treatments on antioxidant activity in Pear cv. “Huang guan” during storage. *Food Bioprocess. Technol.* 7, 671–681. doi: 10.1007/s11947-013-1085-9

Husa, L., L, Wang, S., Zhang, Y., Guo, R. Z., Wu, M. S., Chen, Q., et al. (2014b). Effects of chitosan and calcium chloride treatments on malic acid-metabolizing enzymes and the related gene expression in post-harvest pear cv. “Huang guan.” *Sci. Hort.* 165, 252–259. doi: 10.1016/j.scienta.2013.10.034

Iglesias, A. A. N. A., Shaara, S. A. M. A., Elkhishen, M. A., and Elsherbini, N. R. M. (2017). Pre-harvest application of calcium chloride and chitosan on fruit quality and storability of “Early Swelling” peach during cold storage. *Ciência Agrot.* 41, 220–231. doi: 10.1590/1413-70542017142005917

Jiang, X., Lin, H., Shi, J., Neethirajan, S., Lin, Y., Chen, Y., et al. (2018). Effects of a novel chitosan formulation treatment on quality attributes and storage behavior of harvested litchi fruit. *Food Chem.* 252, 134–141. doi: 10.1016/j.foodchem.2018.01.095

Jongp, P., Rojsitthisak, P., Wangsomboondee, T., and Seraypheap, K. (2017). Influence of chitosan coating combined with spermidine on anthracnose disease and qualities of ‘Nam Dok Mai’ mango after harvest. *Sci. Hortic.* 224, 180–187. doi: 10.1016/j.scienta.2017.06.011

Khalifa, I., Barakat, H., El-Mansy, H. A., and Soliman, S. A. (2016). Improving the shelf-life stability of apple and strawberry fruits applying chitosan-incorporated oil processing residues coating. *Food Packag. Shelf Life* 9, 10–19. doi: 10.1016/j.fpfl.2016.05.006

Khalifa, I., Barakat, H., El-Mansy, H. A., and Soliman, S. A. (2017). Preserving apple (Malus domestica var. Anna) fruit bioactive substances using olive wastes extract-chitosan film coating. *Innov. Food Agric.* 4, 90–99. doi: 10.1002/Infa.2016.11.001

Lei, J., Yang, L., Zhan, Y., and Caner, C. (2016). Potential of antimicrobial active packaging “containing natamycin, nisin, pomegranate and grape seed extract in chitosan coating” for preserving quality and increases stilbene content. *Sci. Hortic.* 176, 425–431. doi: 10.1016/j.scienta.2017.06.011

Li, H., Wang, Y., Liu, F., Yang, Y., Wu, Z., Cai, H., et al. (2015). Effects of chitosan, calcium chloride, and pullulan coating treatments on antioxidant activity in Pear cv. “Huang guan” during storage. *Food Bioprocess. Technol.* 7, 671–681. doi: 10.1007/s11947-013-1085-9

Li, H., Wang, Y., Liu, F., Yang, Y., Wu, Z., Cai, H., et al. (2015). Effects of chitosan on control of postharvest blue mold decay of apple fruit and the possible mechanisms involved. *Sci. Hortic.* 186, 77–83. doi: 10.1016/j.scienta.2015.02.014

Liu, K., Yuan, C., Chen, Y., Li, H., and Liu, J. (2014). Combined effects of ascorbic acid and chitosan on the quality maintenance and shelf life of plums. *Sci. Hortic.* 176, 45–53. doi: 10.1016/j.scienta.2014.06.027

Lopes, U. P., Zambolim, L., Costa, H., Pereira, O. L., and Finger, F. L. (2014). Potassium silicate and chitosan application for gray mold management in strawberry during storage. *Crop Prot.* 63, 103–106. doi: 10.1016/j.cropro.2014.03.013

Lu, L., Liu, Y., Yang, J., Araz, R., Yu, T., and Zheng, X. (2014). Quaternary chitosan oligomers enhance resistance and biocontrol efficacy of *Rhodotorula paludigenum* to green mold in satsuma orange. *Carbohydr. Polym.* 113,174–181. doi: 10.1016/j.carbpol.2014.06.077
Ma, Z., Yang, L., Yan, H., Kennedy, J. F., and Meng, X. (2013). Chitosan and oligochitosan enhance the resistance of peach fruit to brown rot. Carbohydr. Polym. 94, 272–277. doi: 10.1016/j.carbpol.2013.01.012

Mohammadi, A., Hashemi, M., and Hosseini, S. M. (2015). Nanoencapsulation of Zataria multiflora essential oil preparation and characterization with enhanced antifungal activity for controlling Botrytis cinerea, the causal agent of gray mould disease. Innov. Food Sci. Emerg. Technol. 28, 73–80. doi: 10.1016/j.ifset.2014.12.011

Moussa, S. H., Tayel, A. A., Alsohmi, A. S., and Abdallah, R. R. (2013). Botrytis cinerea-induced spoilage. Sci. Hortic. 224, 367–373. doi: 10.1016/j.scienta.2017.06.046

Tayel, A. A., Moussa, S. H., Salem, M. F., Mazrou, K. E., and El-Tras, W. F. (2016). Control of citrus molds using bioactive coatings incorporated with fungal chitosan/plant extracts composite. J. Sci. Food Agric. 96, 1306–1312. doi: 10.1002/jsfa.7223

Treviño-Garza, M. Z., García, S., Flores-González, M., del, S., and Árvalo-Niño, K. (2015). Edible active coatings based on pectin, pullulan, and chitosan increase quality and shelf life of strawberries (Fragaria × ananassa). J. Sci. Food. 80, M1823–M1830. doi: 10.1111/1750-3841.12938

Valenzuela, C., Tapia, C., López, L., Bunter, A., Escalona, V., and Abugoch, L. (2015). Effect of edible quinoa protein-chitosan based films on refrigerated strawberry (Fragaria × ananassa) quality. Electron. J. Biotechnol. 18, 406–411. doi: 10.1016/j.ejbt.2015.09.001

Velikova, E., Winkelhausen, E., Kuzmanova, S., Alves, V. D., and Moldão-Martins, M. (2013). Impact of chitosan-beeswax edible coatings on the quality of fresh strawberries (Fragaria × ananassa cv Camarosa) under commercial storage conditions. Food Sci. Food Technol. 52, 80–92. doi: 10.1016/j.lwt.2013.02.004

Waezthongkrak, W., Puschpen, S., and Leelasuphakul, W. (2015). Effect of Bacillus subtilis and chitosan applications on green mould (Penicillium digitatum Sacc.) decay in citrus fruit. Postharv. Biol. Technol. 99, 44–49. doi: 10.1016/j.postharvbio.2014.07.016

Wang, Y., Li, B., Zhang, X., Peng, N., Mei, Y., and Liang, Y. (2017). Low molecular weight chitosan is an effective antifungal agent against Botryosphaeria sp. and preservative agent for pear (Pyrus) fruits. Int. J. Biol. Macromol. 95, 1135–1143. doi: 10.1016/j.ijbiomac.2016.10.105

Wu, T., Dai, S., Cong, X., Liu, R., and Zhang, M. (2017). Succinylated soy protein film coating extended the shelf life of apple fruit. J. Food Process. Preserv. 41, 13024–13034. doi: 10.1111/jfpp.13024

Xoca-Orozco, L. A., Cuellar-Torres, E. A., González-Morales, S., Gutiérrez-Martínez, P., López-García, U., Herrera-Estrella, L. et al. (2017). Transcriptomic analysis of avocado Hass (Persea americana Mill) in the interaction system fruit-chitosan- Colletotrichum. Front. Plant Sci. 8:956. doi: 10.3389/fpls.2017.00956

Zhang, H., Li, R., and Liu, W. (2011). Effects of chitin and its derivative chitosan on postharvest decay of fruits: a review. Int. J. Mol. Sci. 12, 917–934. doi: 10.3390/ijms12020197

Zhang, W., Li, L., Pan, S., Liu, M., Zhang, W., Liu, H., et al. (2017). Controls postharvest decay and elicits defense response in kiwifruit. Food Bioprocess. Technol. 11, 1937–1945. doi: 10.1007/s11947-017-1957-5

Zhou, Y., Zhang, L., and Zeng, K. (2016). Efficacy of Pichia membranaefaciens combined with chitosan against Colletotrichum gloeosporioides in citrus fruits and possible modes of action. Biol. Control 96, 39–47. doi: 10.1016/j.biocontrol.2016.02.001

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