### IMPROVING FRUIT QUALITY AND STORABILITY USING POSTHARVEST TREATMENTS WITH BENEFICIAL MICROBES AND NATURAL COMPOUNDS – AN OVERVIEW

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#### Abstract

Fresh fruits are very perishable and susceptibile to damage very quickly after harvest during storage with significant losses of quality characteristics and thus of the yield. Chemical fungicides were intensively used to reduce the incidence of post-harvest diseases, maintaining quality and extend shelf life, but they led to the development of resistance to various pathogens and the appearance of residues on the fruit surface, which represents a risk for consumers. To minimize storage losses, a varied range of postharvest treatments have been evaluated to reduce fungal disease and extend the storage period of the fruits while maintaining the quality. The present review provides a brief overview on the use of different postharvest treatments with natural compounds and/or beneficial microorganisms and summarises information about their effect on maintaining quality, antioxidant capacity and reduce fungal diseases during fruit storage.

Key words: fungal disease, storage, Trichoderma, chitosan, salicylic acid, essential oils.

#### INTRODUCTION

Fruits are an important part of the human diet because they are a source of soluble sugars, minerals, and dietary fibers. Nowadays other properties of fruits are gaining importance, such as their antioxidant potential due to a wide range of bioactive compounds and enzymes with health benefits in decreasing the risk of developing cancer and cardiovascular diseases, among others. Some of these compounds are polyphenols (including anthocyanins), carotenoids, vitamin C, and tocopherols, which are present in varying concentrations in fruits.

Due to their perishable nature, fruits deteriorate very quickly after harvest with significant loss of quality characteristics and thus production. During the storage period, due to various internal and external factors, chemical, physical and physiological changes that occur in fresh fruit can lead to significant losses of nutritional and sensory quality and production. Various postharvest treatments can be applied to maintain fresh quality with high nutritional value and meet the safety standards of fresh produce. These postharvest treatments are generally combined with appropriate management of storage temperatures (Mahajan et al., 2014). In this context, in order to maintain the nutritional quality but also the bioactive compounds and the antioxidant capacity during fruit storage, the purpose of this study is to new and identify effective post-harvest compounds treatments with natural or beneficial microorganisms.

### Effect of salicylic acid application on fruit quality during storage conditions

In recent years, salicylic acid (SA) has been widely used to maintain postharvest quality, delay fruit ripening, and increase nutritional value during storage (Chen et al., 2023; Supapvanich and Promyou, 2013). Being a natural hormone, salicylic acid is considered a safe compound as a post-harvest treatment (Asghari and Aghdam, 2010).

As recent research reports show, salicylic acid can improve fruit physical properties such as size, weight (Shafiee et al., 2010) and firmness, soluble sugars, and acidity (Shafiee et al., 2010; Zhang et al., 2003; Srivastava and Dwivedi, 2000). Da Rocha Neto et al. (2016) also support the positive effect of maintaining the quality of apple fruits treated post-harvest with salicylic acid.

Salicylic acid was very effective in delaying fruit ripening and senescence (Mohammadi and Aminifrad, 2013), thus increasing fruit keeping capacity by decreasing weight loss, maintaining firmness (Khademi and Ershadi, 2013) along with improving peach fruit health. Similarly, Shafiee et al. (2010) noted that salicylic acid was able to reduce fruit weight loss while maintaining firmness and color.

Also, salicylic acid treatments applied to apples during storage reduce electrolyte leakage, prevent loss of firmness, and maintain the characteristics of acidity, vitamin C, soluble sugars along with increased carotene and anthocyanin content compared to the untreated control.

Previous research suggests that exogenous application of salicylic acid can decrease metabolic rate, delay ethylene production preventing postharvest diseases, and alleviate physiological disturbances such as frost injury in fresh strawberry or pomegranates fruit during storage (Sayyari et al. 2011; Babalar et al., 2007).

Also, salicylic acid induces an increase of bioactive compounds contents and the activity of antioxidant enzymes including catalase, peroxidase, and superoxide dismutase (Ghasemzadeh and Jaafar, 2013; Dokhanieh et al., 2013). The antioxidant activity of peaches treated with SA is significantly reduced during the storage period (Shokri et al., 2020).

The research of the authors Supapvanich and Promyou (2017) showed that the application of a 2.0 mM salicylic acid solution maintains the post-harvest quality of papaya fruits of the 'Kaek Dam' and 'Holland' varieties and any higher concentration can cause damage to the skin of the fruit and susceptibility to fungal attack. Also, the research of Supapvanich and Promyou (2013) suggest that a concentration of 0.5 mM applied to 'Taamtimjaan' apples maintains postharvest quality characteristics such as fruit firmness and freshness during short-term storage. However, exceeding this concentration leads to the appearance of brown spots on the skin of apples.

Some studies have shown that fruits treated with salicylic acid such as cherries (Giménez et al., 2017), peaches (Tareen et al., 2012), and apples (Delijou et al., 2017) show a reduced production of superoxide free radicals and an increased activity of antioxidant enzymes compared to the untreated control during storage.

However, Imran et al. (2007) demonstrated that salicylic acid improved the antioxidant capacity of pears. But, Adhikary et al. (2020) found that pears treated with salicylic acid showed reduced oxidation of phenolic content by inhibiting the action of polyphenol-oxidase, maintaining an increased phenolic content thus leading to an increased incidence of browning. and also maintaining the content of vitamin C and superoxide dismutase activity, these results contradicting the results of Imran et al. (2007). However, pears treated with 2 mM salicylic acid solution exhibited 15% and 20% higher firmness than untreated fruits after 30 respectively 70 days of storage. Similar results were demonstrated by Razavi et al. (2014) in peaches and by Delijou et al. (2017) who reported that post-harvest treatment of fruits maintains firmness during storage.

Recently, Haider et al. (2020), supporting that the post-harvest application of salicylic acid influences antioxidant activity and antioxidant enzymes, but decreases the effect of this treatment on the content of carotenoids, enzymes involved in fruit softening. So, they argue that a solution of salicylic acid in a concentration of 0.002  $\mu$ mol/L applied to 'Kinnow' tangerines variety was very effective in reducing the loss of weight, firmness, juice content and also slowed down the activity of the enzymes involved in the fruit softening maintaining the higher content in soluble sugars, carotenoids and vitamin C extending the storage time at low temperatures.

The relationship between the concentration of salicylic acid applied, the degree of fruit ripening and the activity of enzymes involved in cell wall degradation has been well documented (Zhang et al., 2003). They reported that ethylene production and polygalacturonase, pectinmethylesterase and cellulase activity decreased after postharvest salicylic acid treatment.

## Effect of chitosan/chitin application on fruit quality during storage conditions

Chitosan is a natural polysaccharide, derived from chitin found in the cell wall of pathogenic fungi or shell of crustaceans. Chitosan and its derivatives are able to form semi-permeable films on the fruit surface that can act as a mechanical barrier to protect the fruit from pathogen infection and induce host defense response, decreasing the infection during storage period (Meng et al., 2010; Bautista-Banos et al., 2006) and also regulates the gas exchange and reduces transpiration loses and fruit ripening is slowed down (Jiang & Li, 2001; Du et al., 1997).

The combination between the treatment with chitosan and the storage temperature is associated with a reduced production of  $CO_2$  resulting in a decrease in the fruit ethylene production. (Li & Yu, 2000; Du et al., 1997).

The results of studies by Ali et al. (2011) suggest that chitosan treatment not only maintains papaya fruit firmness but also improves fruit quality during cold storage.

Cui et al. (2020) indicate that the application of a combination of chitosan and salicylic acid before harvest has a more pronounced effect in delaying the ripening of apricots than their individual application. Moreover, the treatment with this combination maintains the quality characteristics of the 'Xiaobai' apricot variety during storage, including the delay in the loss of firmness, the decrease in the content of soluble sugars, acidity and color change. It also increases the frost tolerance of apricots. The effects of chitosan treatment are much more evident in terms of reducing the intensity of respiration and ethylene synthesis when it is applied post-harvest to mandarin and guava fruit (Elmenofy et al., 2021; Baswal et al., 2020).

Chitosan was also able to reduce anthocyanin degradation and prevent pomegranate fruit color deterioration during cold storage (Varasteh et al., 2012). In the same trend, Jiang et al. (2018) reported that a new formulation based on chitosan (Kadozan), led to a reduction in the intensity of fruit respiration, delaying the increase in cell membrane permeability, maintaining an increased content of anthocyanins and thus maintaining quality and extending the shelf life of litchi fruits.

Strawberries showed a reduction of flesh maintain anthocvanins browning and polyphenol content and prolonged storage life after treatment with chitosan (Petriccione et al., 2015b). The importance of postharvest chitosan treatment has also been reported for apricots where increased phenols content and antioxidant enzyme activities (Petriccione et al., 2015a).

The effect of chitin and chitosan has been demonstrated in post-harvest diseases control to maintain quality and extend fruit shelf life. Both substances are effective in reducing postdiseases inhibiting harvest by spore germination and mycelial growth of phytopathogenic fungi due to the formation of a film on the fruit surface.

In recent years, many reports have shown that chitin can increase the effectiveness of yeasts such as *Rhodotorula glutinis*, *Rhodosporidium paludigenum*, to control post-harvest fruit diseases (Lu et al., 2014). The results of Fu et al. (2016) showed that 0.1-1% colloidal chitin solution could effectively inhibit blue rot produced by *Penicilium expansum* in pears without adverse effects on quality, because chitin does not present a risk to human health and for the environment and is widely available in nature, its applications individually may be more economical than in combination with other biocontrol agents for postharvest fruit fungal diseases.

Chitosan induces the biochemical defense response in stored fruits, maintains firmness and moisture and the amount of vitamin C. Chitosan applied individually in 0.5 or 1% concentrations was the most effective against pathogens of stored fruits, but in combination with yeasts such as *Cryptococcus laurentii* in a concentration of 0.1% was effective for *Penicilium expansum* in apples stored at 20°C. Perdones et al, (2012) reported the increased efficacy of the combined application of chitosan with lemon oil for the inhibition of postharvest pathogens and extend strawberries shelf-life. In Romania, the studies of Radu (2012) demonstrated the effectiveness of the application of chitosan film after harvest for maintaining the quality of apples, especially of an increse quantity of soluble sugars in the Generos, Starkrimson and Ionagold varieties and maintaining a high acidity in the Idared and Ionagold varieties.

## Effect of essential oils application on fruit quality during storage conditions

Essential oils are natural substances extracted from aromatic and medicinal plants. These compounds play an important role in fruit preservation, contributing to the safety and extension of the shelf life. They are also nontoxic, hypoallergenic, and safe for consumption (Laranjo et al., 2017).

The effectiveness of essential oils is attributed to the presence of phenolic compounds, terpenes and alcohols being an important and healthy alternative to chemical compounds applied to fruits during storage (Laranjo et al., 2017). The extension of the fruit storage period results from their action by reducing the activity of antioxidant enzymes.

Experiments conducted by Rabiei et al. (2011) reported that post-harvest treatment of apples with thyme and sage oil improved fruit quality after 5 months of storage at low temperature 1°C and relative humidity 90%.

The results of Shehata et al. (2020) indicated that strawberry fruits treated with citrus essential oils (lemon, orange, tangerine) have a content of antioxidants higher and phytochemical compounds than untreated fruits, due to the protective effect against molds. It is noteworthy that the application of all citrus oils extended the storage period and delayed the deterioration of strawberry fruits up to 18 days, maintaining the content of soluble sugars, acidity and anthocyanin content.

Recently, Cai et al. (2020) developed starch films impregnated with thyme essential oil that by applying them to mango fruits during storage found that it inhibited fruit weight loss, reduce vitamin C loss, and delayed quality changes related to mango fruit ripening. Essential oils are a source of antimicrobial bioactive compounds, which can be used for plant protection with strong antifungal and antibacterial effects. Romanian researchers have used essential oils with an antimicrobial effect especially as antifungal agents for cereals during storage, and also to stimulate germination in different cereals (Dudoiu et al., 2017; Fatu et al., 2017). A wide variety of plant volatile compounds and essential oils have been tested for postharvest disease control in fruit and are promising potential antifungal agents for use as biofungicides in fruit storage disease protection (Sivakumar and Bautista-Banos, 2014). Moreover, since essential oils have low toxicity mammals. are biodegradable. to multifunctional. non-persistent in the environment and are easy and cheap to obtain. the possibility of using them in the protection of stored fruits is considered a very attractive idea in recent years (Pandey et al., 2017).

Different studies have explored the potential of essential oils as antifungal agents (Shehata et al., 2020; Cai et al., 2020). Oregano and thyme essential oils have significant antifungal activity against *B. cinerea* and *P. expansum* infection in stored apples (Rabiei et al., 2011).

Despite the antifungal potential at low concentrations of many volatile compounds tested in vitro, commercial implementation is severely limited due to problems related to decreased *in vivo* efficacy, potential phytotoxicity, and low sensory characterization.

The studies carried out in Romania, regarding the behavior of apples from the varieties Idared, Golden Delicious, Starkimson, claimed that those treated with volatile oils and calcium chloride presented a better resistance to the attack of pathogens and also there was a delay in the appearance storage diseases by 1-2 months (Anghel, 2007).

The studies of Cosoveanu et al. (2013) concluded that essential oils, aqueous and alcoholic plant extracts of different species of *Artemisia* and *Argyranthemum frutescens* show antifungal activity against important pathogens during fruit storage such as *P. expansum, Botrytis cinerea, Alternaria alternata.* The results also indicate that the plant extracts used have different antifungal activity depending on the plant species, extract type, concentration and pathogen type.

Also, Groza (2015) studying the effect of some natural products (plant extracts and propolis)

on the fungi *Monilia fructigena* and *Penicilium expansum* in different varieties of apples during storage found that propolis tincture had a fungistatic and fungicidal effect on the colonies of *P. expansum*, however, plant extracts had no effect, the fungus developing immediately. However, both the propolis tincture and the extracts had an inhibitory effect on the colony of *Monilia fructigena*.

# Effect of beneficial microorganisms on the induction of disease resistance in fruits during storage

Bioagents of the *Trichoderma* genus are extremely versatile in inhibiting post-harvest fruit diseases caused by different phytopathogens *Penicilium expansum* (apples, pears), *Botrytis cinerea* (strawberry, table grape) (Batta et al., 2007; Batta, 2004).

Senthil et al. (2011) support that Trichoderma harzianum applied during storage reduces the incidence of blue mould produced hv *P.expansum* in grapes and increases the storage time at low temperatures. El-Katatny and Emam (2012) reported that post-harvest treatments with Trichoderma suspensions in a concentration of  $10^7 - 10^8$  spores/ml increases resistance to Alternaria alternata, Aspergillus spp. and significantly decrease the incidence of the disease in tomatoes. Other studies have post-harvest treatment shown that with Trichoderma in conditioned form leads to a strong reduction in disease intensity caused by blue mold in apples (Quaglia et al., 2011; Batta, 2007). Also, long-lasting protection against infection is obtained and fruit damage is prevented or delayed.

In Romania, there are only a few studies on increasing the systemic resistance of vegetables treated with beneficial microorganisms and highlighting the accumulation of some secondary metabolites as well as the intensification of the activity of antioxidant enzymes. A study on the induction of systemic resistance by Trichoderma strains confirmed the intensification of photosynthesis and the increase in the content of assimilatory pigments in tomato plants and thus the reduction of symptoms caused by Botrytis cinerea infection (Alexandru et al., 2013).

The exploitation of biopreparations based on microbial biocontrol agents in our country was

low due to the low effectiveness compared to chemical fungicides. However, some biopreparations have been realized based on *Trichoderma viride* (Trichosemin 25) used to control gray rot and white rot on sunflower and beans, and based on *Trichoderma viride* (Trichopulvin) used to combat gray rot in grapevine and strawberry (Sesan et al., 1999) which have similar effectiveness to that of chemical products used for the same purpose, presenting the advantage of being classified in the IV toxicity group.

Yeasts have been extensively used to control post-harvest diseases in various fruits, they are safe antagonists, do not produce antibiotics, have no risks and have a low ecological impact, the mechanism of action is based on competition for nutrients and space and by the production of antifungal metabolites (Sharma et al., 2009; Janisiewicz & Korsten, 2002). Induction of resistance in host tissue has been suggested as another mode of action of antagonistic yeasts for inhibition of postharvest diseases in fruit (Sharma et al., 2009; Spadardo et al., 2002).

Numerous yeasts from the genus Cryptococcus, Pichia, Saccharomyces, and Kloeckera have particularly attracted attention because they can colonize the fruit surface for a long period of conditions time; in dry they produce extracellular polysaccharides and can restrict the areas of colonization and germination of fungal propagules, being very little affected by pesticides. Some studies have reported that different strains of Candida saltoana, Candida oleophila, Candida laurentii, and Pichia guilliermondii can induce resistance to numerous postharvest diseases in fruits (Droby et al., 2009).

Wounded (damaged) tissue has been shown to be characterized by a marked presence of reactive oxygen species (ROS) and the ability of antagonistic yeasts to adapt to oxidative stress has been closely related to yeast multiplication in fruit wounds and biocontrol efficacy (Castoria et al., 2003). The increased activity of superoxide dismutase and catalase was closely correlated with the increase in biocontrol efficiency and stress tolerance induced by yeasts, detoxification is dependent on antioxidant enzymes and the increase in their activity is of great importance for the amelioration of oxidative stress.

It is well known that polyphenoloxidase is an important enzyme associated with disease resistance induced by biocontrol yeasts such as Rhodotorula mucilaginosa (Liu et al., 2013), Rhodosporidium paludigenum (Zhu et al., 2013). The results of Lu et al. (2014) showed that polyphenol oxidase activity in apples treated with *R. paludigenum* combined with 1% chitin was significantly higher than that in apples treated with yeast alone, indicating that the increase in enzyme activity may be part of the mechanism by which resistance to blue mold is induced. Addition of chitin to veast suspension increases catalase and superoxide dismutase activity and decreases malondialdehyde content. However, most of the antagonistic yeasts when used individually cannot completely control postharvest fruit diseases like synthetic fungicides, thus, many ways have been proposed to improve the effectiveness by combining with other compounds (Zhu et al., 2013). In the same way, it was shown that chitin in a concentration between 0.5-1% can increase the effectiveness of the antagonistic yeasts Cryptococcus laurentii (Yu et al., 2008).

Also, some yeasts such as *Rhodotorula glutinis*, *Cryptococcus laurenti, Aureobasidium pullulans* actively contribute to the decrease of patulin accumulation in *Penicilium expansum* infected apples during storage, because they can metabolize this mycotoxin. According to Romanazzi et al. (2013), chitosan can control fungal diseases that damage the quality of fruits during storage. The storage time of papaya fruits was extended by 33 days by using the combined treatment between chitosan and calcium chloride (Romanazzi et al., 2017).

### CONCLUSIONS

■ Post-harvest treatment of fruits with different natural compounds and beneficial microbial agents can maintain fruit quality and induce resistance to different pathogens during storage.

■ The natural compounds with a role in maintaining post-harvest fruit quality and inducing resistance to diseases frequently used

are chitin, chitosan, salicylic acid, essential oils, plant extracts,  $\beta$ -aminobenzoic acid.

• The essential oils used to maintain quality and induce post-harvest resistance to fruit are: thyme oil, cinnamon oil, mint oil, clove oil, and oregano oil.

■ From the beneficial microbial agents most often used in the activation of systemic resistance are various fungal strains: *Trichoderma* spp and different yeasts (*Candida saltoana*, *Candida oleophila*, *Candida laurentii*, *Pichia guilliermondii*).

■ Plant defense mechanisms can be enhanced by combined application of microbial biocontrol agents and natural compounds versus their individual application. Also, these combined treatments have a superior effect compared to the application of chemical fungicides in diseases control.

• Post-harvest treatments represent an ecofriendly, safe, non-toxic alternative for the environment and human health, cheap and easy to apply to fruit during storage.

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