Environmentally Friendly and Safe Technologies for Quality of Fruits and Vegetables

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SECTION 4. ENVIRONMENTALLY FRIENDLY AND SAFE METHODS TO CONTROL POSTHARVEST LOSSES

22. NEW DEVELOPMENTS IN ALTERNATIVE METHODS TO CONTROL POSTHARVEST FRUIT DECAY

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Abstract

Public concern in food safety and the increase of pathogen resistant populations has enhanced the interest in developing methods to control postharvest fruit decay alternative to fungicides. According to their nature alternative methods can be classified as biological, chemical or physical. This article reviews research on alternative postharvest disease control methods and explores new possibilities of research to improve their efficacy.

Keywords: alternative methods, biological control, chemical control, integrated approach, physical methods, postharvest control

Introduction

Postharvest decay of fruits and vegetables may reach very important values depending on species, harvest methods, storage, transportation, etc., representing up to 25% of the total production in developed countries and 20-50% in developing countries. Therefore working with efficient methods to reduce losses caused by postharvest pathogens remains a priority.

Postharvest losses can be reduced by preventing fruit infections avoiding fruit by damages, carefully handling, applying correct sanitation procedures and using fungicides. The repeated and continious use of fungicides has led to the development of fungal strains resistant to many fungicides. In addition, the growing concern for human safety and environmental protection, the imperative of sustainable agriculture and development of integrated crop management and organic production have resulted in the need to find other methods to control postharvest decay. According to their nature these alternative methods can be classified as biological, chemical or physical.

The purpose of this manuscript is to review the research work in alternative environmentally friendly and safe approaches to control postharvest diseases of fruits.

Biological Control in Postharvest

Over the past 20 years biological control of postharvest diseases using microbial antagonists has emerged as an effective strategy to control the major postharvest decays of fruits and several reviews have been published (Janisiewicz 1988; Wilson & Wisniewski 1989; Wisniewski & Wilson 1992; Janisiewicz & Korsten 2002; Droby *et al.* 2009; Nunes *et al.* 2009; Sharma *et al.* 2009). During this period several programs worldwide have been carried out to develop microorganisms with antagonistic activity in several fruits, using different strains of bacteria, yeast and filamentous fungi (Table 1).

Postharvest environment represents a particular advantage to develop biological control. Injuries made during harvest and transportation to packinghouse can be protected from wound pathogens with only a single application of the biocontrol product directly to infection site (harvested fruit), using the existing facilities (Janisiewicz & Korsten 2002). During storage, fruits are kept in a constant physical environment, which can be controlled to favour the antagonist growth. The high value of the commodities in postharvest makes the application of a biocontrol fungicide more justified than in the field.

Table 1. Biological control agents of fruit postharvest diseases.

Biocontrol Agent	Disease	Fruit	Ref
Bacteria			
Bacillus amyloliquefaciens	*Colletotrichum musae, Fusarium moniliforme*	banana	Alvindia & Natsuaki 2009
Bacillus licheniformis	*Botryosphaeria* spp., *C. gloeosporioides*	mango	Govender *et al* 2005
Bacillus pumilus	*Penicillium digitatum*	citrus	Huang *et al* 1992;
Bacillus subtilis	*Botrytis cinerea, C. gloeosporioides, Monilinia fructicola, P. digitatum, P. expansum*	apple, avocado, citrus, stone	Korsten *et al* 1995; Sholberg *et al* 1995; Fan *et al* 2000; Leelasuphakul *et al* 2008
Pantoea agglomerans	*B. cinerea, P. digitatum, P. expansum, P. italicum, Monilinia* sp., *Rhizopus stolonifer*	citrus, pome, stone	Nunes *et al* 2001a,2002; Teixidó *et al* 2001; Bonaterra *et al* 2003;
Pantoea ananatis	*P. expansum*	pome	Torres *et al* 2005
Pseudomonas cepacia	*B. cinerea, P. digitatum, P. expansum, Monilinia* sp.	pome, lemon, stone	Janisiewicz & Roitman 1988; Smilanick & Denis-Arrue 1992; Smilanick *et al* 1993
Pseudomonas glathei	*P. digitatum*	citrus	Huang *et al* 1995
Pseudomonas syringae	*B. cinerea, P. digitatum, P. expansum, P. italicum*	citrus, pome	Janisiewicz & Marchi 1992; Bull *et al* 1997; Nunes *et al* 2007a
Serratia plymuthica	*P. digitatum*	orange	Meziane *et al* 2006
Yeast			
Candida famata	*P. digitatum*	citrus	Arras 1996
*Candida guilliermondii**	*B. cinerea, P. digitatum, P. italicum, Geotrichum candidum, R. stolonifer*	apple, citrus, peach	Chalutz & Wilson 1990; McLaughlin *et al* 1992
Candida oleophila	*B. cinerea, G. candidum, P. digitatum, P. expansum*	apple, citrus, stone	Droby *et al* 1998; 2002b
Candida sake	*B. cinerea, P. expansum, R. stolonifer*	kiwifruit, pome	Viñas *et al* 1998; Cook *et al* 1999
Candida saitoana	*B. cinerea, P.digitatum, P. expansum*	apple, citrus	El-Ghaouth *et al* 2000
Cryptococcus laurentii	*Aspergillus niger, B. cinerea, P. expansum, R. stolonifer*	grape, pome, strawberry	Roberts 1990; Lima *et al* 1998
Kloeckera apiculata	*A. niger, B. cinerea, R. stolonifer*	apple, grape, peach	McLaughlin *et al* 1992
Metschnikowia andauensis	*B. cinerea, P. digitatum, P. expansum, P. italicum, R. stolonifer*	citrus, pome	Manso & Nunes 2010
Metschnikowia fructicola	*Alternaria* spp., *A. niger, B. cinerea*	grape	Kurtzman & Droby 2001; Karabulat *et al* 2003
Metschnikowia pulcherrima	*B. cinerea, Monilinia* sp., *P. expansum*	apple, grapes	Piano *et al* 1997; Janisiewicz *et al* 2001
Pichia anomala	*B. cinerea, Botryodiplodia theobromae, C. musae, F. moniliforme*	apple, banana, guava	Jijakli & Lepoivre 1998; Lassois *et al* 2008; Hashem & Alamri 2009
Rhodotorula glutinis	*B. cinerea, P. digitatum, P. expansum*	apple, oranges	Zheng *et al* 2005; Zhang *et al* 2009
Filamentous fungi			
Aureobasidium pullulans	*B. cinerea, M. laxa, P. expansum*	apple, peach, strawberry	Adikaram *et al* 2002; Bencheqroun *et al* 2007; Zhang *et al* 2010
Epicoccum nigrum	*M. laxa*	peach	Madrigal *et al* 1994
Muscodor albus	*B. cinerea, M. fructicola, P. digitatum, P. expansum*	apple, lemon, peach	Mercier & Jiménez, 2004; Mercier & Smilanick 2005
Trichoderma asperellum	*Thielaviopsis paradoxa*	pineapple	Wijesinghe *et al* 2010

* *Candida guilliermondii syn.: Pichia guilliermondii, Debaryomyces hansenii*

Selection of a Postharvest Biocontrol Agent

The first step to develop a biocontrol system is the isolation and screening of a biological control agent (BCA). The fructoplane has been an excellent source of antagonists to control postharvest fruit pathogens. Different strategies have been used to screening BCAs, but since the antagonists are applied in consumable products often they are evaluated on wounded fruits instead of on *in vitro* studies (Nunes *et al.* 2009). The development of a BCA for postharvest diseases is an interactive process with several steps, including tests reflecting packinghouse conditions, enhancement of biocontrol activity, scale-up the production and development of a formulated product of the BCA.

Mode of Action of Postharvest Biocontrol Agents

The mode of action involves a complex interaction between host, pathogen, BCA and environment, comprising process of antibiosis, nutrient and space competition, induced resistance, parasitism and lytic enzymes production. Often more than one mechanism is present.

Competition for nutrients and/or space is reported has the major mode of action of postharvest BCAs (Droby *et al.* 1989; Janisiewicz *et al.* 2000; Nunes *et al.* 2001b; Bencheqroun *et al.* 2007). This hypothesis is supported by the fact that biocontrol activity of an antagonist depends on their concentration in the wound. So it can be considered if a BCA rapidly grows by depleting the available nutrients in the wound, it will prevent the possibility of the pathogen to use these nutrients to germinate and initiate the infection process. In most reports on biological control of postharvest diseases a quantitative relationship has been demonstrated between the BCA concentration in the wound and its efficacy (Vinãs *et al.* 1998; Nunes *et al.* 2002, 2007a).

Antibiosis by antibiotic production has been suggested in part as mode of action of bacteria. However leads us the debate if an antibiotic-producing microorganism should be used in postharvest phase, due to the concern of introducing an antibiotic into food and the possible development of a pathogen resistance. Concerning to parasitism and lytic enzymes production by BCAs, few reports are available, as the attachment of *Pichia guilliermondii* to the mycelium of the pathogen and subsequent changes in hyphae (Arras *et al.* 1998) and changes and degradation of the hyphae and cell walls of *Botrytis cinerea* due to the production of exo-beta-1,3-glucanase by *Pichia anomala* (Jijakli & Lepoivre 1998). Induced resistance in fruits has been observed in the presence of some BCAs, such the increase of ethylene production, phenylamina ammonia lyase (PAL) activity, phytoalexin biosynthesis, accumulation of chitinase and β-1,3-glucanase in fruits (Droby *et al.* 2002b).

Volatile compounds are suggested as a new mode of action and refers the use of antimicrobial volatiles produced by BCAs, and has been introduced as a better alternative because there is no contact with the food and less manipulation of the commodities would be involved. Good candidates for biocontrol by biofumigation are *Muscodor albus* (Mercier & Smilanick 2005) and *Bacillus subtilis* JA (Chen *et al*. 2008).

Enhancement, Development and Commercial Application of Biocontrol

The effectiveness of a BCA when applied alone has been a limitation of its use since is inconsistent and has a narrow range of activity either on fruits and/or diseases. Taking into account the mode of action, the importance of a rapid colonization of fruit wounds by the BCA and the interactions in microbial communities, different approaches could be used to improve and develop new biocontrol systems: (i) BCAs mixture that could increase the spectrum of activity, as the antagonist action will result from the action of a community of microorganisms; (ii) manipulation of nutritional environment in order to make it advantageous to the BCA and/or limited to pathogens; (iii) pre-harvest application that allow the BCA to have longer interact with the pathogen, and colonise tissues before the arrival of pathogen, such as latent infection and incipient infections; (iv) genetic manipulation of BCA, as insertion of genes or over-expression of endogenous genes responsible for antifungal activity or, insertion of genes for better utilization of available nutrients; (v) production process to improve the ecological fitness of BCA and formulation to enhance viability, efficacy and shelf-life of BCA formulated cells, and (vi) integration with physical and/or chemical methods, (will be discussed in this review) taking advantage of the additive or synergistic effects in order to improve the efficacy of each method.

From an industry point of view, a BCA should be able to be produced at a large-scale in a short period of time and as a cost-effective process using as a growth medium by-products from food industries (Hofstein & Chapple 1998). Recently Manso *et al.* (2010) reported high biomass productivity of *Pantoea agglomerans* PBC-1 using by-products from carob industry as carbon source. Other by products, such molasses, malt extract, dry beer extract have been used (Abadias *et al*. 2003). To be commercialized a BCA has to be developed as a formulated product. Postharvest BCAs have been formulated mainly as a refrigerated liquid (Costa *et al.* 2001; Abadias *et al.* 2003), a solid formulation using freeze-drying (Abadias *et al.* 2001) and wettable refrigerated powder (Janisiewicz & Jeffers 1997). All these techniques of formulated cells have particularly effects on cells viability.

Several microorganisms have been reported as BCAs against fruit postharvest diseases however only a few biological products are available in the market: Aspire™ (*Candida oleophila*, Ecogen Inc, USA), Bio-save™ (*Pseudomonas syringae*, Jet Harvest Solutions, USA), Shemer™ (*Metschnikowia fructicola*, Bayer Crop Science, AG), YieldPlus™ (*Cryptococcus albidus*, Anchor Yeast), Avogreen (*Bacillus subtilis*, RE at UP, South Africa) are commercialized in various countries. In Europe there are three more products: Candifruit™ (*Candida sake*, Sipcam-Inagra, Spain), Pantovital (*Pantoea agglomerans*, Biodurcal, Spain) and Boni-Protect® (*Aureobasidium pullulans*, Bio-protect, Germany). The search for new antagonists should be permanent as in chemical industry the search for new molecules are constant, as well the objective to broaden the use of BCAs to different diseases and commodities.

Physical Control

Physical treatments when applied alone have the advantage of produce no residues, providing an environmentally friendly means of postharvest pathogen losses control. The most important and developed physical methods are: heat and UV-C.

Heat Treatments

Heat treatments for control postharvest diseases may be applied to fruit by hot air (curing) or hot water.

Curing treatment is applied by holding fruits at high temperature (30-40 ºC) and high relative humidity (>90%). Can be applied in a cycle of 2-6 days at approximately 30-38 ºC (Fallik *et al.* 1995; Plaza *et al.* 2003), in a shorter period, 0.5-24 h, at higher temperature, >40 ºC (Nunes *et al.* 2007b), or as intermittent treatment with more than one cycle. Pérez *et al.* (2005) effectively controlled decay in citrus applying a curing treatment of 2 cycles of 18 h at 38 ºC with an intermediate period of 6 h at 20 ºC. The effect of curing has been studied in different fruits (Fallik *et al.* 1995; Cook *et al.* 1999; Casals *et al.* 2010; Wang *et al.* 2010) and extensively in citrus fruit (Ben-Yehoshua *et al.* 1987; Rodov *et al.* 2000; Plaza *et al.* 2003; Pérez *et al.* 2005; Nunes *et al.* 2007b). In fact the most common use of curing to control decay is in citrus, and was first reported in the first half of 20th Century. Commercial use is not frequent as is expensive, at low temperature required long time and at higher temperature have some risks, such heat phytotoxicity and weight loss.

Hot water treatments can be applied by brief dippings (0.5-5 min) in water at 45-55 ºC or by hot water drench (20-60 s at 55-65 ºC) (Palou *et al.* 2002a; Smilanick *et al.* 2003; Torres *et al.* 2007). A more recent technique is the use of hot water sprays at 50-70 ºC during 10-60 s over rotating brushes (Porat *et al.* 2000; Karabulat *et al.* 2002). Among heat treatments the use of hot water is preferred because water is a more efficient heat transfer medium, easier to use, require shorter period of treatment, technology is cheaper and is easier to combine with other alternative methods. Hot water has been tested either alone or in combination with other alternative methods in a huge range of type of fruits: table grapes (Gabler *et al.*

2005), cherries (Karabulat *et al.* 2004a), stone fruit (Karabulat *et al.* 2002), litchi (Olesen *et al.* 2004) and again in more extend in citrus (Porat *et al.* 2000; Palou *et al.* 2002a; Torres *et al.* 2007).

The effect of heat is direct to the pathogen, by inhibition of spore germination and mycelium development, and indirect by induction host resistance to the pathogen. The inhibition of pathogens is affected by different factors. For example Barkai-Golan (2001) reported that germinated spore are more sensitive than ungerminated spores; in other work Sommer *et al.* (1967) showed that *Monilia fructicola* is more sensitive than *Botrytis cinerea*, B. *cinerea* than *Rhizopus stolonifer*, and *Penicillium expansum* is more tolerant than these species. Plaza *et al.* (2003) reported a 90% reduction of green and blue mould decay in oranges treated with curing at 33 °C for 65 h, but total control of both pathogens was observed with treatments at 40 °C for 18 h (Nunes *et al.* 2007b). Apart from species, physiological state, temperature and duration of treatment, the effect of treatment depends in other factors, such, moisture content, metabolic activity, age of inoculums, etc. (Ben-Yehoshua & Porat 2004).

The effect of heat treatment in induction of resistance by the host has been attributed to physical changes in the epiticular surface of fruits, such closing cuticle fractures by melting natural peel waxes, the enhancement of antifungal activity, induction of heat-shock proteins and pathogenesis-related (PR) proteins.

UV-C Illumination

Application of UV-C at low doses (180-280 nm) is known to reduce postharvest decay in several fruits (Stevens *et al.* 1996; Nigro *et al.* 1998; Marquenie *et al.* 2003; Cia *et al.* 2007). Despite the fact that UV-C direct inhibit the pathogen by DNA damages the major mode of action of UV-C is elicitation of resistance in fruits (Droby *et al.* 1993; Shama & Alderson 2005). As heat treatments, UV-C at 248 nm has shown to elicit production of phytoalexins (Kim *et al.* 1991), lignin-like compounds on fruit peel and PR proteins (Pombo *et al.* 2009), induction of PAL or peroxidase enzymes (Droby *et al.* 1993; El-Ghaouth *et al.* 2003). Other beneficial effects in fruit quality induced by the abiotic stress caused by UV-C should be taking into account, since all these changes in chemistry of fruits could enhance their nutraceutical value (Cisneros-Zevellos 2003). Erkan *et al.* (2008) reported the increase of antioxidant enzyme activity in strawberry after treatment with UV-C, and Dong *et al.* (1995) the increase of anthocyanins in apples.

Shama & Alderson (2005) made a compilation of several works in UV-C treatments, and reported treatment doses of UV-C to control decay in a range of 0.5 to 15 kJ m^2 depending on fruit and pathogen. However due to undesire effects such UV-C phytotoxicity the intensity of the treatment should be carefully monitored.

The use of UV-C treatment in fruits could become a commercial practice. There are some patents and commercial prototypes, however more research is needed since the response of the product to UV-C treatments is specific for each commodity and depends among other factors, on the level of maturity, temperature of storage and the side of fruit that have been exposed to the illumination.

Other Physical Methods

Pulsed light, ionizing radiations and microwaves are other physical methods to control postharvest decay. Pulsed light is a relatively recent technology, using short time pulses of intense broad spectrum rich in UV-C, with only a few reports of its use in postharvest (Lagunas-Solar *et al.* 2006). Ionizing radiation has a relatively short wavelength and high energy that allow high penetration and effectiveness. To be applied in fruits ionizing radiation can be produced by Gamma rays, X-rays or electrons beams. The use in fruits and vegetables was approved by United States Food and Drug Administration (US-FDA) at doses up to 1,000 Gy. As it was previously discussed for physical treatments, the effect of ionization radiation depends on type of radiation and energy level, type of fruit, maturity stage, temperature, etc. (Kader 1999). The effects of these treatments are also direct and indirect depending on the type of radiation; however it is not clear for each treatment which is the most important effect. The application of ionization radiation has limitations, mainly due to the acceptance of the consumers and the high cost of equipments and operations.

Chemical Control

There are several chemical alternative control methods to postharvest fungicides, however to be considered as a safe alternative should have minimal toxicological effects.

GRAS Substances

Generally Regarded as Safe, GRAS, is a classification of the US-FDA and is recognized among experts as safe under the conditions of its intended used.

Bicarbonate and carbonate salts are widely used as food additives with no restrictions for many applications by European and USA regulations. It has been found to have antimicrobial activity. Among them, to control postharvest decay, focus have been made to sodium bicarbonate (SBC; $NaHCO_3$) and sodium carbonate (SC; Na_2CO_3), especially to control postharvest decay of citrus fruit (Smilanick *et al.* 1995, 1997, 1999), but also in other fruits such grapes (Gabler & Smilanick 2001), melon (Aharoni *et al.* 1997), banana (Alvindia & Natsuaki 2007), etc. The mode of action of these salts is unclear but appears to be primarily fungistatic and not very persistent (Smilanick *et al.* 1999). SBC and SC are inexpensive, readily available, and can be used with a minimal risk of injury to the fruit, and when solutions are heated their activity is greatly enhanced.

Ethanol occurs in many food products and additives, and it was approved for use as a disinfectant or sanitizer USDA. Ethanol, as liquid or vapour treatment, has been reported to be effectively to control postharvest decays especially of table grapes and against several pathogens such *B. cinerea*, *Alternaria alternata*, and *Aspergillus niger* (Karabulat *et al.* 2004a,b; Gabler *et al.* 2005), but also to control postharvest diseases of stone and citrus fruit (Smilanick *et al.* 1995; Margosan *et al.* 1997).

Ozone (O_3) was declared as GRAS for food contact in 1997 and since that the interested in its application has been increased. Ozone is a natural substance in the atmosphere and one of the most potent sanitizers against a wide spectrum of microorganisms (Khadre *et al.* 2001). O_3 can be easily produced *in situ* and applied as a gas (continuous or intermittent exposure) or dissolved in water without leaving any residue since their product of degradation is oxygen. To control postharvest decays, O_3 has been applied in citrus, stone, strawberry and table grapes (Pérez *et al.* 1999; Palou *et al.* 2001, 2002b; Smilanick *et al.* 2002; Gabler *et al.* 2010). However there are several reports demonstrating the lack of effect of ozone (Palou *et al.* 2001). The application of ozone in air has some risks for human health, so requires some protective measures to workers.

Natural Compounds

Plants produce a wide range of secondary metabolites (e.g. essential oils, alkaloids, phenols, flavonoids) biologically actives with antifungal properties and with low toxicity to mammals and safe for environment. Some of them are recognize as GRAS compounds. Among secondary metabolites, flavour compounds have distinctive properties to be used in postharvest, such volatility, low- water solubility and easily adsorption. Special interest has been addressed to essential oils. The activity of essential oils in control postharvest pathogens in vitro was reported in different studies (Arras & Usai 2001; Plaza *et al.* 2004a; Viuda-Martos *et al.* 2007) however their effects rarely have been demonstrated in fruit at concentrations similar to *in vitro*. In general, the essential oils that showed greatest antifungal postharvest activity were from thyme (thymol and carvacrol), cinnamon and clove. Interest has been driven to the natural compounds and essential oils of citrus fruit, as will be discussed in a manuscript in this book by Rodov *et al.* The commercial application could be interesting however much more investigation is needed: the mode of action of essential oils is not fully explained but has been attributed to the damage of the membrane structure (Moleyar & Narasimham 1987), and many problems of phytotoxicity are associated.

Chitosan is a soluble form of chitin, normally obtained from crustacean shells, with antifungal properties and the capacity to induce resistance in plants at low concentration (approximately 1%). Chitosan has been showed to control decay in apple, table grape, strawberry, banana and citrus (El-Ghaouth *et al.* 1992;

Capdeville *et al.* 2002; Chien *et al.* 2007; Romanazzi *et al.* 2007). The primarily antifungal mechanism of chitosan is believe to be the direct effect in pathogen, although some works report the induction of host resistance (Capdeville *et al.* 2002).

Integrated Control

This review reported the extensive research and significant progress made worldwide in the last decades in postharvest diseases of fruits. There are already several non-fungicidal methods at a commercial stage with ability to control of several diseases in different crops. However to be commercially successful, any product/ technology to be used in the postharvest phase have to control diseases in more than 95%. Therefore one approach to use these methods as an alternative to synthetic fungicides is an integrated strategy, taking advantage of the additive or synergistic effects of different treatments in order to overcome the performance and improve the efficacy of each one.

Some examples are the combination of biocontrol agents with physical and/or chemical treatments. Positive synergistic effect occurred with the combination of *P. agglomerans* followed by curing treatment at 33 °C for 6 h in controlling green mould in lemons (Plaza *et al.* 2004b). Application in citrus fruit of *P. agglomerans* after treatment with solutions of heated sodium bicarbonate allowed a control similar or superior to imazalil (Torres *et al.* 2007; Usall *et al.* 2008). Enhancement of biocontrol activity was achieved in pome fruits combining L-serine, L-aspartic acid or ammonium molybdate with *Candida sake* (Nunes *et al.* 2001b, 2002), in papaya using sodium bicarbonate and *Candida oleophila* (Gamagae *et al.* 2004). Other compounds such sugar analogs, calcium salts, organic acids have been combined with biological methods to manage postharvest decay control (Janisiewicz *et al.* 1998; Karabulut *et al.* 2001; Nunes *et al.* 2001b; Ippolito *et al.* 2005). A result of this integrated approach of biocontrol systems is the development of a second generation of products such "Biocoat" whose main components are *Candida saitoana* and chitosan or "Biocure" also with *C. saitoana* and lysozyme. Both products contain other additives such sodium bicarbonate (Wisniewski *et al.* 2007).

Another important approach has been the combination of different physical and/or chemical treatments, in most of the cases including heat, specially the use of heated chemical solutions. As already reported the heating solution of sodium carbonate or bicarbonate significantly enhanced their effectiveness (Smilanick *et al.* 1997; Palou *et al.* 2002a). The use of heated ethanol has been tested with success for control postharvest decay in citrus, peaches, table grapes and sweet cherry (Smilanick *et al.* 1995; Karabulat *et al.* 2004a,b; Gabler *et al.* 2005) in a concentration between 10-30%. The combination of these two treatments allowed a reduction in concentration of ethanol and temperature improving not only the efficacy of treatments but also safety issues and minimizing injuries of the product. The efficacy of organic acids was also enhanced in the control of *P. expansum* on apples and *P. digitatum* on oranges when applied as heated solutions (Salazar *et al.* 2007)

Several works report other combinations using alternative methods, including UV-C, white light and heat (Marquenie *et al.* 2003), X-ray and sodium bicarbonate (Palou *et al.* 2007), chitosan and ethanol (Romanazzi *et al.* 2007), plant extracts, chitosan and heat (Win *et al.* 2007) etc.

Conclusions

In this review several safe and environmentally friendly treatments alternative to conventional fungicides were reported as effectively to control postharvest decays in fruits, and, in general, with effectiveness comparable to fungicides. Research in biological control has made important advances allowing the development of some commercial products, showing a promising and new alternative system. Physical methods have several advantages such a total lack of residues, presenting often direct and indirect effect, and some of them have a simple and inexpensive application. Chemical methods use natural or synthetic compounds with low toxicity, like GRAS compounds. They are in general inexpensive, readily available, and suitable for the postharvest handling practices. Although the high efficacy of the treatments the specificity host/pathogen, the cost, the lack of curative effects and the high variability represent a restriction to their implementation. To overcome these shortcomings, the combination of these methods as an integrated strategy demonstrates to be viable approach alternative to synthetic fungicides.

In the future the commercial use of these methods will be very important thus organic producing are growing worldwide, since the use of synthetic fungicide is not allowed. Furthermore, nowadays some supermarkets already demands for fruits and vegetables free of residues of postharvest products, because the chemical residues are more likely to be present when fruits will be consumed. Research should be driven to large scale trials, to understand the host/pathogen interaction and validate the viability of the implementation of these practices in packinghouses.

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