Environmentally Friendly and Safe Technologies for Quality of Fruits and Vegetables

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The papers contained in this book report some of the peer reviewed Proceedings of the International Conference "Environmentally friendly and safe technologies for quality of fruit and vegetables", but also other papers related with the subject were included. The manuscripts were reviewed by the Editor and Editorial Board, and only those papers judged suitable for publication were accepted. The Editor wish to thank to all the reviewers and authors for their contribution.

Authors are responsible for content and accuracy of their papers.

Proceedings of the International Conference "Environmentally friendly and safe technologies for quality of fruit and vegetables", held in Universidade do Algarve, Faro, Portugal, on January 14-16, 2009. This Conference was a join activity with COST Action 924.

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

27. SUGAR VARIATION IN HEALTHY, BLUE MOLD INFECTED AND *AUREOBASIDIUM PULLULANS* TREATED 'ROCHA' PEAR

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Abstract

Soluble sugars variation in 'Rocha' pear was studied in healthy, infected with *Penicillium expansum* (blue mold) and treated with the antagonist *Aureobasidium pullulans* fruits. Pears from four pickings were analyzed after 1, 3 and 5 months of cold storage (-0.5 ºC, 95% RH). For each storage period fruits were inoculated and kept at room temperature (*ca*. 20 ºC) for 5 d and analyzed. After 1 month of cold storage sucrose increased from the first to the last picking, while fructose, glucose and sorbitol were stable in healthy fruits. After 5 d at room temperature non-inoculated fruits presented fructose and glucose rises and sorbitol decreases for the 4 harvest dates, while sucrose increased in earlier yielded fruits but decreased in the last two pickings. After infection with *P. expansum*, in general, was observed a tendency to decrease in all sugars. The application of the antagonist *A. pullulans* partly reverses such tendency. The antagonist alone causes lower disturbances in sugar contents, except in glucose that may present slight decreases. Considering only the fruits of the commercial harvest date (DC3), sugars tend to increase along cold storage (except sucrose), particularly in healthy fruits and for most cases of both blue mold and antagonist inoculated fruits. On the other hand, the tendency for sugar decrease in *P. expansum* infected fruits, reported for 1 month of cold storage, is still detectable for glucose and sucrose after 3 months, and glucose, fructose and sorbitol after 5 months. After 3 and 5 months of storage, the maintenance of sugar content in fruits of the DC3 inoculated with both blue mold and the antagonist was not as clear as for 1 month. Data suggested that cold storage was beneficial for sugar increase of healthy fruits, except for sucrose. In the DC3 fruits, sugar loss caused by *P. expansum* was higher after 1 month of storage, but the use of the antagonist *A. pullulans* partly reverses that tendency.
Keywords: Antagonism, biocontrol, soluble sugars

Introduction

´Rocha' is a Portuguese pear cultivar that shows important losses caused by postharvest infections with *Penicillium expansum* Link (blue mold), usually initiated in wounds occurring during harvest and packing. Synthetic fungicides are usually used to control pathogens, but its application has become more limited due to health concerns and contamination by chemical residues (Ragsdale & Sisler 1994). Also, many fungicides are loosing their effectiveness due to the development of resistance by many pathogens.

Various control methods have been investigated with promising results but none, alone, was as effective as fungicides. Biological control is an alternative to chemical control that shows promising results in the control of postharvest diseases (Janisiewicz *et al.* 2001). Among the microorganisms tested, the yeast-like fungus *Aureobasidium pullulans* showed antagonistic activity in apple (Ippolito *et al.* 2000) and in 'Rocha' pear (Borges *et al.* 2004) fruits infected by *P. expansum*. In 'Rocha' pears, the highest efficacy was observed at the commercial harvest date, decreasing thereafter during cold storage of fruits. That activity was improvement by calcium treatments and when chemically complemented with low doses of Imazalil (Barreiro *et al.* 2006). Understanding the mode of action of biocontrol agents is important to improve their performance; competition for space and nutrients, antibiosis and activation of host defenses are the main antagonistic activities exerted (Castoria *et al.* 2001).

Sugars are indicators of metabolic activity in fruits and quantitative changes can result in flavor changes. In pears, sorbitol performs the function of a reducer, regulates coenzymes activity and may play a role in osmoregulation of water stress resistance. Moreover, sorbitol synthesis appears always as an additional metabolic path to sucrose synthesis (Hudina *et al.* 2000).

The objectives of this study were the elucidation of a possible involvement of some sugars in the biocontrol of *P. expansum* by *A. pullulans* and the evaluation of sugars changes in healthy, infected and treated 'Rocha' pears.

Material & Methods

Fruits

'Rocha' pears were grown under a program of Integrated Fruit Production in an orchard of Estação Nacional de Fruticultura Vieira Natividade (ENFVN), Portugal. Fruits were weekly hand-harvested at 4 dates, including commercial one (DC3), and cold stored (*ca.* -0.5 ºC and 95% RH) at COOPVAL until use. Pears were disinfected with 0.5% NaClO solution, water rinsed and air dried prior to the infection assays.

Pathogens

Isolates of *P. expansum* were obtained from infected 'Rocha' pear and maintained on PDA. The pathogen was incubated at 25 ºC for 7 days (d). Spore suspensions (3.1×10^4 conidia mL^{-1}) were prepared as described by Janisiewicz & Marchi (1992).

Antagonist

A. pullulans was isolated from 'Rocha' pear, leaves and fruits in the ENFVN orchard. The antagonist was cultured on PDA at 25 ºC for 7 d. Subsequently the cultures were flooded with sterile distilled water and diluted to a concentration of 3.2×10^8 cfu mL^{-1} for biocontrol assays.

Biocontrol Assays

Infections were performed in 1, 3 and 5 months cold stored fruits, left at room temperature overnight (Barreiro *et al.* 2006). 'Rocha' pears were wounded twice (each wound 1.5 cm distant from the equator of the fruit, with a depth of 4 mm and a diameter of 4 mm) and inoculated with 25 µL of an aqueous suspension of *A. pullulans* and/or 20 µL of an aqueous suspension of *P. expansum*. The following treatments were performed: fruits receiving 25 µL of sterilized water (C), infected with *P. expansum* (P), treated with *A. pullulans* (A), pretreated with *A. pullulans* and infected 3 h later with *P. expansum* (A+P). Fruits were analysed 5 d after the treatments.

Sugar Analysis

Beside the mentioned treatments (C, A, P, A+P), also healthy pears (H) immediately after cold storage were used. In infected fruits, healthy tissues below wounds were collected with a cork borer, weighed, frozen in liquid N_2 and kept at -80 ºC until analysis. The analyses were performed according to the method of Hudina & Stampar (2000). Sugar separation and quantification were performed in 20 µL aliquots, using an HPLC Waters (USA) equipped with a RI Detector (2414 Waters), a Sugar-Pak1 Column (300×6.5 mm, Waters), at 90 ºC, with H_2O (containing 50 mg EDTA-Ca L^{-1}) as elluent and a flow of 0.5 mL min^{-1}. For sugar identification and quantification were used known standards.

Statistical Analysis

Analysis of variance (ANOVA) was applied to the results and a Tukey's test for mean comparison (95% confidence level) was used.

Results & Discussion

The efficacy of *A. pullulans* to control *P. expansum* postharvest infections of 'Rocha' pears was already shown (Borges *et al.* 2004; Barreiro *et al.* 2006). It was found that sugars changed not only during ripening, (when fruits were taken from cold storage and kept at *ca*. 20 °C for 5 d), but also due to pathogen infection and antagonist application.

Results presented in Fig 1 and 2 shows that fructose is the major soluble sugar. In fact, fructose, due to its restricted phosphorylation, is a less active precursor in fruit carbohydrate metabolism than glucose. That facilitates its accumulation in the vacuole of the parenchyma cells in apple (Berüter *et al.* 1997) leading to its high value.

Furthermore, healthy fruits (H) analyzed immediately after 1 month of cold storage (Fig 1) showed a sucrose increase during the picking period (DC1 to DC4), probably a consequence of starch breakdown (data not shown) in the later stages of fruit development. The conversion of starch into sucrose by sucrose synthase, as found in pears (Moriguchi *et al.* 1992), contributes to fruit sweetening (Beruter & Feusi 1997).

Stable values of fructose and sorbitol were observed along the picking dates in H fruits, while glucose presented a slight (non-significant) tendency to decrease.

When H fruits were kept for 5 d at *ca*. 20 °C (C) some changes occurred. In general, glucose, fructose and sucrose (the latter only in DC1 and DC2) increased, while sorbitol decreased. In fact, sorbitol seems to be an important C-source for hexose production during fruit maturation (Berüter & Feusi 1997). Thus, sorbitol oxidation after 5 d at 20 °C (C) could have contributed to fructose and glucose increase, as reported also in apples (Berüter & Feusi 1997; Berüter *et al*. 1997). Along the picking dates, fructose had a slight increase between H and C, while glucose presented increasing differences between H and C going from DC1 to DC4.

In fruits inoculated with *P. expansum* analyzed after cold storage (1 month) followed by 5 d at *ca*. 20 °C (P), the fructose and sorbitol contents (as well as in glucose and sucrose in some DCs) were lower than in healthy fruits under the same conditions (C) (Fig 1). The application of the antagonist together with *P. expansum* (A+P) reverses that tendency for most of the cases where P provoked a decrease compared to C.

Fruits from commercial harvest (DC3), cold stored for 1, 3 and 5 months (Fig 2) showed a decrease of sucrose during storage. These decreases may have contributed to increase fructose and glucose content, as observed along the storage period in all treatments (Berüter & Feusi 1997). Sorbitol also increases. On the other hand, the decrease of sugars in *P. expansum* infected fruits (P) as compared to C, observed after 1 month of cold storage, is still detectable for glucose and sucrose after 3 months and for glucose, fructose and sorbitol after 5 months. In most cases, the sugar content was not influenced by the application of the antagonist followed by the pathogen (A+P), when compared to the C treatment.

In general, as expected, cold storage favored the increase of sugars except sucrose that decreased. Keeping the fruit at 20 °C for 5 d was beneficial for sugar increment in healthy fruits, except for sorbitol that might have been used for sucrose synthesis and afterwards for monosaccharide increment. Late harvest (DC4) was beneficial for glucose content in healthy and infected fruits kept 5 d at room temperature after cold storage, while sorbitol slightly decreased. Fruits inoculated with *P. expansum* only in some cases, showed loss of sugars. However, the application of the antagonist *A. pullulans* previous to the inoculation with *P. expansum* helped (in most cases) the maintenance of sugar levels closer to those of healthy fruits.

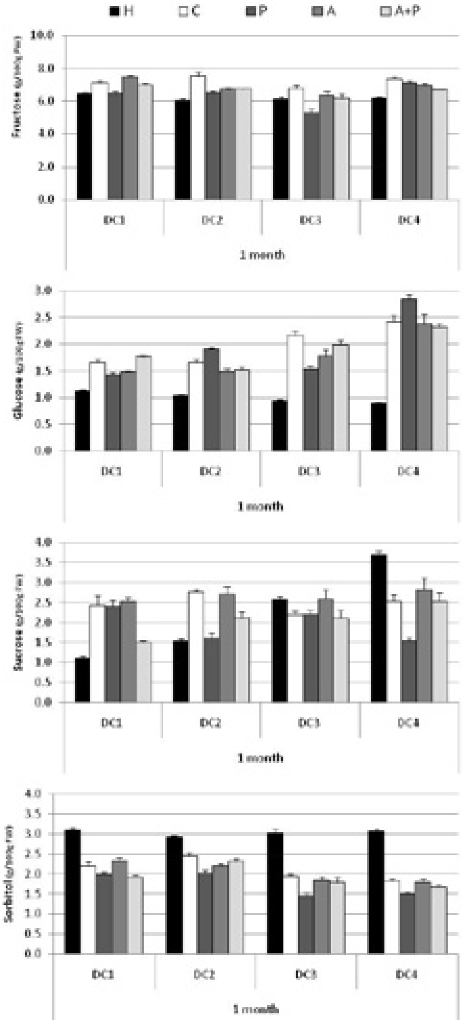

Fig 1. Sugar variation along weekly picking dates (DC1 to DC4) after 1 month of cold storage. Healthy fruits were analyzed immediately after cold storage (H), after 5 d at 20 ºC (C), after infection with *P. expansum* (P), after treatment with *A. pullulans* (A) after treatment with *A. pullulans* followed by infection with *P. expansum* (A+P). Bars represent the mean ± SE (n=10 fruits).

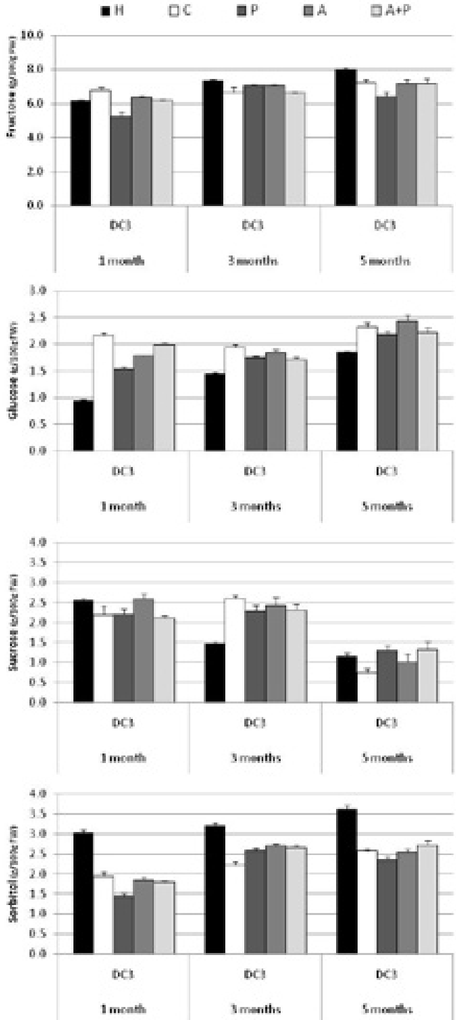

Fig 2. Sugar variation in fruits of the commercial harvest date (DC3) along the cold storage period (1, 3 and 5 month). Healthy fruits were analyzed immediately after cold storage (H), after 5 d at 20 ºC (C), after infection with *P. expansum* (P), after treatment with *A. pullulans* (A) after treatment with *A. pullulans* followed by infection with *P. expansum* (A+P). Bars represent the mean ± SE (n=10 fruits).

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