Postharvest Biology and Technology 144 (2018) 36–45

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Impact of *Pseudomonas graminis* strain CPA-7 on respiration and ethylene production in fresh-cut 'Golden delicious' apple according to the maturity stage and the preservation strategy

Cyrelys Collazo[a], Jordi Giné-Bordonaba[b], Ingrid Aguiló-Aguayo[b], Ismael Povedano[a], Dolors Ubach[b], Inmaculada Viñas[a,*]

[a] Food Technology Department, University of Lleida, XaRTA-Postharvest, Agrotecnio Center, Rovira Roure 191, 25198, Lleida, Catalonia, Spain
[b] IRTA, XaRTA-Postharvest, Edifici Fruitcentre, Parc Científic i Tecnològic Agroalimentari de Lleida, Parc de Gardeny, 25003, Lleida, Catalonia, Spain

ARTICLE INFO

Keywords:
Antagonist
Fresh-cut fruit physiology
Passive modified atmosphere packaging
MAP

ABSTRACT

The effect of the biocontrol agent (BCA) *Pseudomonas graminis* CPA-7 on the accumulation of CO_2 and ethylene (C_2H_4) in fresh-cut apples at two maturity stages was evaluated in refrigerated conditions. Factors involved in the preservation strategy applied upon commercial conditions such as the antioxidant (AOX) treatment and the storage system were included in the analysis. Regardless of the maturity stage, the BCA reduced C_2H_4 levels within the MAP atmosphere in AOX-untreated apples wedges, by 29 and 43% in immature and mature apples, respectively. Nevertheless, the addition of ascorbate as antioxidant counteracted this effect. In vitro tests suggested that the reduction of C_2H_4 levels was not associated to the uptake of this molecule by CPA-7. Interestingly, in non-inoculated samples AOX treatment showed contradictory effects on C_2H_4 production in MAP conditions by significantly reducing C_2H_4 levels in immature apples (by 23%) while increasing it in mature ones (by 40%). Similarly, CPA-7 had opposite effects on the CO_2 accumulation pattern depending on the storage system or the fruit maturity stage. In this sense, CPA-7 was associated to a higher fruit respiratory activity at an advanced maturity stage yet without inducing the fruit fermentative metabolism or altering the fruit quality during a week of refrigerated storage. Overall, these results show that CPA-7 may contribute to the maintenance of the microbiological and physicochemical quality of fresh-cut apple by modulating the fruit ethylene production and/or respiration.

1. Introduction

The effect of the application of antagonists on fresh-cut produce in commercial conditions is influenced by intrinsic factors like the type and maturity stage of the commodity and extrinsic factors such as temperature, preservative treatments as well as oxygen and carbon dioxide concentrations within packages. From the physiological stand, processed products essentially behave as wounded tissues where the disruption of cell compartmentalization leads to the mixture of cellular components with an increase of enzymatic and respiratory activities as well as an elevated production of ethylene (C_2H_4) (Hodges and Toivonen, 2008; Mahajan et al., 2014). C_2H_4 is, indeed, the main hormone controlling ripening in clicmateric fruit (Reid et al., 1973) and its biosynthesis involves the transformation of S-adenosylmethionine into the precursor 1-aminocyclopropane (ACC) mediated by the enzyme ACC synthase (ACS). ACC is later converted to ethylene by ACC oxidase (ACO) (Yang and Hoffman, 1984). In apple, as a fruit with a climacteric behavior, the regulation of these two steps is auto-inhibitory during fruit development prior to ripening and auto-stimulatory at the onset of ripening (Tatsuki et al., 2007; Wang et al., 2009; Lelièvre et al., 1997). Processing implies the mechanical injury of fruit tissues which induces the activity and synthesis of ACS leading to the formation of "wound ethylene" whose accumulation may be enough to activate the clicmateric phase depending on the size and permeability of packages (Lamikanra, 2005; Yu & Yang, 1980). However, the low availability of ACO in pre-climacteric apples is a limiting factor which modulates ethylene production upon cutting due to their reduced capability for the conversion of ACC into ethylene (Lara and Vendrell, 2000). In the post-clicmateric stage the capacity for ethylene production is also reduced and response to wounding is more limited than in the clicmateric stage (Abeles et al., 1993). Therefore, the physiological stage of the commodity constitutes a key factor to take into account during the production of fresh-cut fruit products (Toivonen and Dell, 2002).

Respiration also shows a biphasic rise during the development of

* Corresponding author.
E-mail address: ivinas@tecal.udl.cat (I. Viñas).

https://doi.org/10.1016/j.postharvbio.2018.05.010
Received 26 March 2018; Received in revised form 15 May 2018; Accepted 16 May 2018

clicmateric commodities, the first one early in development and the second one during ripening or senescence. The second peak usually precedes the autocatalytic ethylene synthesis stage (Fonseca et al., 2002). Moreover, respiration is also induced by cutting due to the loss of compartmentalization of the enzymes involved in the respiration pathways and its substrates, and the activation of key regulatory steps of glycolysis and tricarboxylic acid cycle (Rolle and Chism, 1987). Moreover, the mechanical injury of cell membranes activates the enzymatic degradation of its lipid components, with the formation of long-chain fatty acids whose α-oxidation also causes a rise in respiration (Rolle and Chism, 1987). It is also well stablished that 'wound ethylene' induces fruit respiration (Yu and Yang, 1980). Furthermore, an increase in CO_2 production also occurs in fresh-cut tissues due to the activation of cell repair processes, not only for obtaining energy but for the synthesis of replacement structural compounds (Gomez-Lopez, 2012). The accelerated oxidative breakage of organic substrates and the loss of structure of membranes entailed by the above mentioned processes are detrimental to the nutritional properties and the general quality of fresh-cut fruit (Soliva-Fortuny & Martín-Belloso, 2003).

To reduce both respiration and ethylene production several methods comprising chilling conditions and modified atmosphere packaging (MAP) are amongst the most currently used in the fresh-cut produce industry (Rupasinghe and Yu, 2013). The addition of biocontrol agents (BCA) such as *Pseudomonas* spp. is another method that could contribute to modulate ethylene levels thereby extending the shelf-life of fresh-cut ready-to-eat products. Mechanisms for the modulation of plant ethylene metabolism by *Pseudomonas* spp. have been already documented and may imply its exacerbation or its reduction (Fatima & Anjum, 2017; Glick, 2014; Hase et al., 2003). To accomplish the first mentioned effect, pseudomonads enhance the plant capacity to transform the precursor ACC into ethylene, inducing the expression of C_2H_4-responsive genes (Hase et al., 2003). Consequently, systemic induced resistance (ISR) is triggered or primed allowing plants to respond better to a subsequent infection by a broad spectrum of pathogens (Van Wees et al., 1997). The C_2H_4 reducing effect has been observed in plants upon treatment with pseudomonads with ACC deaminase (ACD) activity (Hernández-León et al., 2015; Singh et al., 2015). ACD cleaves ACC into ammonia and α-ketobutyrate (Honma and Shimomura, 1978) lowering the amount of available ACC and therefore limiting ethylene synthesis (Glick, 2014). As a consequence of this process pseudomonads can delay ripening and senescence, promote growth, prime resistance mechanisms and alleviate deleterious ethylene-mediated plant stresses (Eckert et al., 2014; Glick, 2005; Wang et al., 2000). Belonging to this bacterial group is *Pseudomonas graminis* CPA-7, a whole apple epiphyte biopreservative strain which controls foodborne pathogens such as *Listeria monocytogenes*, *Escherichia coli* and *Salmonella enterica* on fresh cut fruit (Alegre et al., 2013a, b; Abadias et al., 2014; Collazo et al., 2017) and modulates oxidative metabolism in fresh-cut apple (Collazo et al., 2018). In an attempt to clarify its mode of action we also investigated the possibility for CPA-7 to modulate the ethylene metabolism in fresh-cut apple thereby influencing the defense response and/or the senescence of this fruit. With this in mind, we monitored the effect of the antagonist in fresh-cut apples as affected by several factors involved in production and commercial conditions (antioxidant treatment, packaging headspace gas composition and the maturity stage of the commodity). In addition, to assess the effect of CPA-7 metabolic activity in supplying exogenous ethylene or metabolizing the produced by the fruit, the ability of the antagonist to either produce or consume C_2H_4 was tested in vitro.

2. Materials and methods

2.1. Antagonist inoculum preparation

For inoculum preparation, *P. graminis* CPA-7 (Alegre et al., 2013b) was grown overnight in 50 mL of tryptone soy broth (TSB, Biokar, Beauveais, France) at 25 °C in agitation. Cells were harvested by centrifugation at 9800 x g for 10 min and suspended in deionized sterile water. The concentration of the suspension was checked by viable plate count of appropriate ten-fold dilutions in saline peptone (8.5 g L^{-1} NaCl, 1 g L^{-1} peptone) onto TSA plates after incubation at 30 °C for 48 h.

2.2. Fruit processing

Apples (*Malus domestica* Borkh. cv. 'Golden delicious') used in this study were grown in local farms (Lleida, Catalonia, Spain) and collected in august, 2017 at two maturity stages (with a week of difference between harvests). Prior to experimental assays, apples were washed with running tap water, surface disinfected with 700 mL L^{-1} ethanol and either stored as such or processed (peeled with an electric fruit peeler and cut into eight wedges with a handheld corer/slicer). Wedges were kept in chilled (5 °C) chlorinated tap water (pH 6) until treatment and/or packaging.

2.3. In vitro analysis of ethylene production or consumption by CPA-7

2.3.1. Preparation and inoculation of liquid culture media

In vitro assays were performed in order to evaluate the putative ethylene production or consumption by CPA-7 in a culture medium with a similar composition to the fruit but discarding the changes due to the apple's native microbiota. For that, analysis glass tubes containing 10 mL of sterile apple juice were inoculated with CPA-7 to a concentration of 10^5 CFU mL^{-1}. Additionally, aliquots of TSB were prepared, inoculated and analyzed in the same way, to serve as a control treatment. For juices preparation, apple wedges were previously dipped in 6% NatureSeal® AS1 solution (AS1, AgriCoat Ltd., Great Shefford, UK), a calcium ascorbate-based product, or in cold deionized water, for 2 min in agitation (15.7 rad s^{-1}) in a tabletop orbital shaker (Unimax 1010, Heidolph, Germany). Then, juices were obtained in a commercial blender, subsequently filtered through cloth gauzes and either adjusted to pH 6.5 with 1 mmol L^{-1} NaOH or sterilized as such at 215 °C for 5 min and stored at 5 °C until use. Non-inoculated aliquots of each culture medium were used as controls. Inoculated and non-inoculates cultures were stored in agitation in aerobic conditions for 7 d at 5 °C in the case of TSB and apple juice pH 4,5; and at 5 °C or at 25 °C in the case of apple juice pH 6.5. In vitro assays were repeated twice and included three replicates per treatment.

2.3.2. In vitro microbial dynamics

CPA-7 population dynamics in each culture medium was tracked by viable plate count on TSA at 0, 1, 3, 6, and 7 d post-inoculation, as described in Section 2.1.

2.3.3. In vitro CO_2 accumulation pattern

The headspace O_2 and CO_2 composition of each culture tube was measured at 0, 1, 3, 6, and 7 d post-inoculation using a handheld gas analyzer (CheckPoint O_2/CO_2, PBI Dansensor, Denmark). Before each measurement tubes were hermetically closed for 12 h. CO_2 accumulation was expressed in mg mL^{-1} liquid culture medium.

2.3.4. In vitro ethylene accumulation pattern

The ethylene accumulation patterns of cultures tubes previously sealed for 12 h were determined at 0, 1 and 3, 6 and 7 d post-inoculation. At each time,1 mL of gas sample was withdrawn with a syringe from each jar or tray and injected into a gas chromatograph (Agilent Technologies 6890, Wilmington, Germany) fitted with a FID detector and an alumina column F1 80/100 (2 m × 1/8 × 2.1,Tecknokroma, Barcelona, Spain). The injector and detector were kept at 180 °C and 280 °C, respectively. Quantification was carried out by comparing the gas chromatography signal of the samples to that of a 21 μL L^{-1} C_2H_4 standard (Carburos metálicos SL, Aragón, Spain).