LWT - Food Science and Technology 65 (2016) 725–730

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Dynamic controlled atmosphere for prevention of internal browning disorders in 'Rocha' pear

Teresa Deuchande [a], Susana M.P. Carvalho [a, b], Umbelina Guterres [b], Fernanda Fidalgo [c], Nelson Isidoro [d], Christian Larrigaudière [e, **], Marta W. Vasconcelos [a, *]

[a] CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquiteto Lobão Vital, Apartado 2511, 4202-401 Porto, Portugal
[b] Faculdade de Ciências, Universidade do Porto, Departamento de Geociências Ambiente e Ordenamento do Território, Rua do Campo Alegre 697, 4169-007 Porto, Portugal
[c] Biosystems & Integrative Sciences Institute (BioISI), Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal
[d] Coopval, Cooperativa Agrícola dos Fruticultores do Cadaval, Estrada Nacional 115, km 26, 2550-108 Cadaval, Portugal
[e] IRTA, Postharvest Department, Parc Cientific i Tecnològic Agroalimentari, Parc de Gardeny, Edifici Fruitcentre, 25003 Lleida, Spain

ARTICLE INFO

Article history:
Received 8 April 2015
Received in revised form
28 August 2015
Accepted 29 August 2015
Available online 3 September 2015

Keywords:
Ascorbic acid
Fermentative metabolites
Low O_2
Physiological disorders
Pyrus communis

ABSTRACT

This study aimed to evaluate the potential of two dynamic controlled atmospheres, DCA-CF (chlorophyll fluorescence sensor) and DCA-EtOH (ethanol sensor) when compared to controlled atmosphere (CA), in the prevention of internal browning disorders (IBD) in 'Rocha' pear stored under commercial conditions. Pears harvested at optimal maturity were stored for 145 days at −0.5 °C and 95% relative humidity, under three atmospheres: (1) CA (3 kPa O_2 + 0.5 kPa CO_2), (2) DCA-CF and (3) DCA-EtOH. At the end of storage, fruits in DCA-CF did not develop IBD while fruits in DCA-EtOH had an IBD incidence of 15 and 20% after 125 and 145 days of storage, respectively. The higher incidence of IBD under DCA-EtOH may be related to the higher levels of fermentative metabolites and to the lower ascorbate content. In contrast, the higher levels of ascorbate in DCA-CF showed that this technology contributes to maintaining the fruit's antioxidant potential. Collectively our results suggest that DCA-CF is an effective strategy to prevent IBD in 'Rocha' pear. On the contrary, the DCA-EtOH is not suitable to prevent the induction of fermentation and IBD development. The results also suggest that the IBD development in 'Rocha' pear is related to fermentative metabolism.

1. Introduction

'Rocha' pear (*Pyrus communis* L. cv. Rocha) is a Portuguese native variety with a Protected Designation of Origin (PDO) and one of the few export-oriented products of Portuguese agriculture. 'Rocha' pear can be stored for up to 10 months under CA (2–3 kPa O_2 + 0.5–0.7 kPa CO_2 at −0.5 °C and 95% relative humidity), but it is known to be susceptible to internal browning disorders (IBD) and superficial scald, the major causes of postharvest losses during long-term storage under CA (Silva, Gomes, Fidalgo, Rodrigues, & Almeida, 2010). The diphenylamine (DPA) and ethoxyquin as synthetic antioxidants were applied to pome fruits to prevent these physiological disorders during long term storage. However, the current restrictions on their use may have a significant impact on 'Rocha' pear exportation potential. Hence, there is a need to find novel strategies for long term storage of 'Rocha' pear.

The incidence of IBD is mainly related to the gas concentrations in the CA chamber. The reduced O_2 levels can lead to hypoxia within the fruits causing oxidative stress and inducing fermentation. Also, the high levels of CO_2 have been shown to further induce the development of IBD (Deuchande, Fidalgo, Larrigaudière, & Almeida, 2012; Deuchande, Fidalgo, Vasconcelos, Costa, & Larrigaudière, 2015). IBDs are firstly related to membranes damage which may occur as a result of the shift from a predominantly aerobic to an anaerobic metabolism. This metabolic shift may lead to: 1) a decreased energy assumption making it insufficient for cell regeneration and maintenance of the antioxidant system (Franck

* Corresponding author.
** Corresponding author.
E-mail addresses: christian.larrigaudiere@irta.cat (C. Larrigaudière), mvasconcelos@porto.ucp.pt (M.W. Vasconcelos).

http://dx.doi.org/10.1016/j.lwt.2015.08.075
0023-6438/

et al., 2007; Veltman, Lenthéric, Van Der Plas, & Peppelenbos, 2003); and 2) accumulation of fermentative metabolites (ethanol and acetaldehyde), which at high concentration may be toxic to the cells (Chervin, Brady, Patterson, & Faragher, 1996; Ho, Verlinden, Verboven, & Nicolaï, 2006; Ke & Kader, 1992; Ke, Van Gorsel, & Kader, 1990). These factors together may contribute to loss of cell compartmentalization and consequent development of IBD.

Dynamic controlled atmosphere (DCA) aims to provide optimal storage conditions improving the quality and preventing the development of physiological disorders. During storage under DCA, the concentration of O_2 is gradually reduced to the lower limit tolerated by the fruit (less than 1 kPa), the anaerobic compensation point (ACP), below which the respiratory metabolism switches from aerobic to anaerobic. When the critical level of O_2 is reached, the O_2 concentration is automatically or manually adjusted in order to restore optimal storage conditions. The critical level of O_2 is determined by monitoring the physiological fruit responses to stressful low O_2 levels in the storage atmosphere, through the use of specialized sensors. These sensors must be sufficiently precise and reliable to ensure that the atmosphere is adjusted before irreversible fruit damage occurs. Currently, there are three methods capable of detecting significant changes in the following parameters: (1) respiratory quotient (ratio of CO_2 produced/O_2 consumed) (Gasser, Eppler, Naunheim, Gabioud, & Bozzi Nising, 2010); (2) chlorophyll fluorescence (Prange, Delong, Leyte, & Harrison, 2002, 2003) and (3) ethanol production (Schouten, Prange, Verschoor, Lammers, & Oosterhaven, 1997; Veltman, Verschoor, & Van Dugteren, 2003). Prange et al. (2002, 2003) and DeLong, Prange, Leyte, and Harrison (2004) suggested the use of chlorophyll fluorescence sensors as a quick and non-destructive method to determine the minimum acceptable level of O_2 for storing fruits and vegetables, leading to the development of the HarvestWatch™ system for use in CA rooms. DeLong, Prange, and Harrison (2007) demonstrated for two varieties of apples, 'Cortland' and 'Delicious', that storage under DCA using the HarvestWatch ™ system maintained the fruit quality better than storage under CA and reduced the incidence of superficial scald. The same effect was reported for 'Abbé Fétel' and 'Conference' pears (Folchi, Bertolini, & Mazzoni, 2015; Rizzolo, Buccheri, Bianchi, Grassi, & Vanoli, 2015; Vanoli, Rizzolo, & Grassi, 2015). Nevertheless, the storage of 'Abbé Fétel' pears under DCA-CF was reported to induce the incidence of soft scald compared to CA and air storage (Folchi et al., 2015; Vanoli et al., 2015).

Schouten et al. (1997) reported that the storage of 'Elstar' apples in DCA-EtOH led to a better maintenance of the quality attributes than storage under ULO (1.2% O_2 + 2.5% CO_2). Veltman, Verschoor, et al. (2003) have also demonstrated for the same apple variety, that storage under DCA-EtOH contributes to a greater firmness and colour retention after 7 months of storage plus 10 days at room temperature when compared to the CA storage, also leading to a decreased incidence of 'skin spots' a specific physiological disorder of 'Elstar' apple. However, even during storage under air conditions or under levels of O_2 above the lower limit tolerated by the fruits, sometimes there are production of fermentative metabolites, which means that ethanol may not be a reliable indicator to detect changes on the respiratory metabolism (Peppelenbos & Oosterhaven, 1998). Furthermore, in order to ensure reliable ethanol measurements, allowing an adequate adjustment of the O_2 levels in the CA room as function of fruit responses, destructive sampling of the fruit is required.

Regarding 'Rocha' pear, to the best of our knowledge, the use of DCA for long term storage has not been studied yet. Given the beneficial effects of storage under DCA reported for other fruits, this study aimed at evaluating the potential of this technology monitored by two types of sensors (ethanol and chlorophyll fluorescence) in the prevention of IBD in 'Rocha' pear. Emphasis was given to assess the effect of DCA on IBD development and the relationships with the levels of fermentative metabolites and ascorbate during storage. To the best of our knowledge, this is the first time that such an assessment is reported in commercial scale units.

2. Materials and methods

2.1. Plant material and experimental design

Pears (*P. communis* L. cv Rocha) were collected from one orchard located in Cadaval (39° 16′ N, 9° 8′ W). As this experiment was conducted at commercial scale and it was intended to store the fruits for an extended time period, optimally harvested fruits, which have been shown to be less susceptible to IBD (Deuchande et al., 2012), were selected to ensure fruits' marketability upon storage. The fruits were stored in three commercial CA chambers under the following conditions: (1) CA (3 kPa O_2 + 0.5 kPa of CO_2); (2) DCA monitored by a chlorophyll fluorescence sensor (DCA-CF) using an HarvestWatch™ system and (3) DCA monitored by an ethanol biosensor (Tectronick, Senzytech, Italy) (DCA-EtOH). The storage conditions were equally settled concerning temperature (−0.5 °C), relative humidity (95%) and CO_2 concentration (0.5 kPa). The gas composition in the CA chambers was carefully controlled and checked with centralized analysers, supervised by special Fruit Control Equipment software for storage under DCA-EtOH and by an Isolcell software for storage under CA and DCA-CF, both systems with set points and alarms.

The capacity of the chambers used for CA and DCA-CF storage was 150 ton while that used for DCA-EtOH was 400 ton. All the chambers were fully loaded with 'Rocha' pear and were gas-tight allowing levels of O_2 as low as 0.2 kPa to be reached inside the storage rooms. In the two DCA chambers the O_2 levels were adjusted manually according to the physiological fruit responses monitored by the respective sensors. In the DCA-CF chamber the levels of O_2 were reduced to 1 kPa and subsequently 0.2 kPa every two days until the chlorophyll fluorescence peak was detected. Once detected a buffer of 0.2 kPa O_2 was added to the atmosphere until the fluorescence signal was reduced to its initial level. During the remaining storage period the levels of O_2 were adjusted following chlorophyll fluorescence signal (Fig. 1A).

In the DCA-EtOH chamber the pull-down strategy was the same as for DCA-CF and the atmosphere was adjusted to maintain the ethanol level below 20 μL L^{-1}, which was defined as the critical level. At the beginning of storage fruit ethanol was measured every two days and then every ten days. After set point adjustments the levels were measured every two days until a reduction below the established threshold was observed.

Since the fruits were stored in commercial chambers, the main doors of all chambers were opened between the 75 days and 125 days of storage to verify the condition of the fruit and therefore there was an O_2 peak between those time points (Fig. 1).

In each chamber, batches of 70 fruits were placed in front of the inspection port-holes in order to easily remove themat the sampling time points avoiding changes in the storage atmosphere. Periodically, during the storage the incidence and severity of IBD were evaluated and samples of pear pulp for the analyses of ascorbate (3 replicates of 3 fruits each) and pear juice (3 replicates of 10 fruits each) for the analysis of fermentative metabolites (ethanol and acetaldehyde) were prepared. The pulp samples were frozen in liquid nitrogen and ground in a commercial grinder and then stored at −80 °C until analysis. The pear juices were prepared using a commercial blender and filtered through cellulose paper filter and stored at −25 °C until analysis.