Postharvest Biology and Technology 121 (2016) 165–170

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Identification of open lenticels in apples after harvest in relation to lenticel breakdown development during storage

Vikram Singh[a], Dan Gamrasni[b,c], Ruth Ben Arie[b], Shaul Naschitz[d], Haya Friedman[a,*]

[a] Department of Postharvest Science of Fresh Produce, Agricultural Research Organization (ARO), The Volcani Center, Bet Dagan, Israel
[b] Fruit Storage Research Laboratory, Israel Fruit Growers' Association, Kiryat Shmona, Israel
[c] Tel Hai Academic College, Israel
[d] Beresheet Fruit, Kibbutz Iftah, Israel

ARTICLE INFO

Article history:
Received 17 March 2016
Received in revised form 5 June 2016
Accepted 6 June 2016
Available online 4 July 2016

Keywords:
Calcium
Lenticel breakdown (LB)
Open lenticels
SO_2 treatment

ABSTRACT

Lenticel breakdown (LB), appearing as dark brown pits on apple skin, occurs following storage, and the factors associated with LB have not yet been identified. It was assumed that open lenticels at harvest and following postharvest treatments contribute to this phenomenon and therefore a method was developed using SO_2 treatment, to detect air-exposed tissue in Red Delicious apples. The efficiency of detection was assessed on artificial openings, created by pricking the apple surface, which caused bleaching around these openings with enlargement of their dimensions. Similarly, SO_2 treatment of intact apples caused a bleaching around few of the lenticels, indicating that the underlying parenchyma cells were exposed to air. The percentage of open lenticels decreases after harvest during cold storage. A calcium chloride, but not potassium chloride, immersion at harvest resulted in significantly more SO_2 damaged lenticels, indicating that calcium enhances lenticel opening. There was a high correlation between the percentage of open lenticels at harvest and the severity of LB after storage (Pearson's correlation coefficient of $r^2 = 0.80$). This suggests that LB might occur due to exposure of parenchyma cells to air at harvest. In conclusion, these results indicate that the SO_2 treatment at harvest might be used as an efficient method for the prediction of lenticel damage after storage.

1. Introduction

Lenticels are small lens shaped natural openings in the cuticle, appearing consistently on the surface of many fruits. Lenticels in apples (*Malus domestica*) are derived mainly from a gradual disintegration of stomata during fruit development or by the removal of trichomes, mostly in young fruit (Scora et al., 2002). Cracks in the epidermis and cuticle contribute to lenticel enlargement. Lenticels have been categorized into open and closed types (Clements, 1935). Open lenticels result from stomatal modification with large intercellular spaces in sub-stomatal cells or by breakdown and extension of closed lenticels, occurring due to tension at the surface of the growing fruit. Closed lenticels result from covering of open lenticels with external layers of cuticle and wax or by internal development of a phellogen layer in the sub-lenticular cells, responsible for suberin deposition, which blocks the exposure to air of sub-epidermal tissue.

Lenticel breakdown (LB) is a physiological disorder of stored apples cultivars, which may cause considerable postharvest economic losses to growers (Kupferman, 2009). The damage appears as dark superficial pits, which differ from bitter pit and lenticel blotch pit, in that the latter penetrate into the pulp (Anon, 2016http://entomology.tfrec.wsu.edu/Cullage_Site/Physiol_BP.html). The cause and development of LB are not yet well understood, but preharvest environmental conditions prevailing in the orchard, as well as the minerals composition of the fruit, are considered to be possibly involved (Curry, 2003).

Adequate calcium content in apple fruit is necessary to maintain shelf life (Moggia et al., 2006), firmness (Fallahi et al., 2006) and to reduce physiological disorders such as bitter pit and lenticel blotch pit (Raese and Drake, 2002; Casero et al., 2010). Calcium treatment is often applied to maintain fruit quality during storage (Ferguson et al., 1999; Wang and Long, 2015), and can be applied by various means, such as field sprays, postharvest dipping and vacuum infiltration (Roy et al., 1996; Casero et al., 2010). Deleterious effects of calcium have rarely been investigated and to

Abbreviations: SEM, Scanning electron microscopy; LB, Lenticel breakdown.

* Corresponding author at: Department of Postharvest Science of Fresh Produce, ARO, the Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel.
E-mail addresses: hayafrab@gmail.com, hayafr@agri.gov.il (H. Friedman).

http://dx.doi.org/10.1016/j.postharvbio.2016.06.004
0925-5214/

the best of our knowledge, there is only one study (Sharples and Johnson, 1976), which reported that dipping apples in calcium chloride increased LB during storage. Nevertheless, the question of phytotoxicity of calcium applications arises in practical preharvest applications (Techflo, 2013). On the other hand, potassium application to apple has been shown effective to reduce fruit quality (Sharples, 1973; Terblanche et al., 1980).

Sulfur dioxide (SO_2) fumigation is an effective and routinely used postharvest treatment to control disease and improve storage quality of fruit such as longan (Liu, 1999), apricot (Sen et al., 2015) and berry (Cantina et al., 2012). However, SO_2 fumigation often adversely affects fig and litchi fruit quality, because it can penetrate through cracks or wounds in the cuticle, oxidizing and bleaching the surrounding area (Sivakumar et al., 2010; Cantin et al., 2011). Likewise, it can also penetrate through open lenticels in apples (Bompeix, 1972; Amiri and Bompeix, 2005).

The objective of the present study was to detect open lenticels in Red Delicious apples at harvest and to examine their possible contribution to LB during storage. It was postulated that open lenticels at harvest can lead to LB development during storage and might be revealed by SO_2 application after harvest. Open lenticels were also detected by SO_2 following a postharvest treatment with calcium, which is sometimes applied to improve fruit quality in storage.

2. Material and methods

2.1. Fruit material and treatments

Apples (*Malus domestica* cv. Red Delicious) were sampled at commercial maturity stage based on firmness and starch degradation, (the average firmness was 71 N ± 5 SE and starch index was 5.0 ± 1.3 SE) from an orchard in the Upper Galilee, Israel. The experiments were performed in three consecutive years on Red Delicious (2013–2015) and on Gala (2015). Calcium and potassium treatments were applied 2–7 d after harvest by dipping the apples in calcium chloride ($CaCl_2$, 0.14 mol L^{-1}) or potassium chloride (KCl, 0.28 mol L^{-1}) for 5 min. Control apples were not dipped. The experimental design was 4 replicates of 40 fruit per treatment, from which individual fruits were sampled randomly at each examination for each of the various tests. Fruits were allowed to dry at room temperature for 2–3 h and analyzed subsequently, following SO_2 treatment (see below). In experiments where the combined phytotoxic effect of calcium and SO_2 was investigated, the effects of $CaCl_2$ and KCl at the above concentrations were examined.

2.2. Sulfur dioxide (SO_2) treatment

Apples were subjected to SO_2 treatment after being pricked, to determine the effect on artificially exposed tissue, or remained unpricked, to detect natural openings. The artificial openings were created with a needle, by randomly pricking 20 holes/apple of approximately 1.0 mm in diameter and 2 mm in depth, on the entire surface. The SO_2 treatment was performed by placing the apples in netted nylon bags in an air-tight container, containing 0.15 g of sodium metabisulfite ($Na_2S_2O_5$) per liter volume of container in a separate dish, from which SO_2 was released by wetting the sodium metabisulfite with a minimal volume of water. Fruits were left undisturbed for 6 h at room temperature and then were aerated for 24–48 h at room temperature. Control fruits were held under similar conditions without treatment. Artificial openings were categorized into 4 types based on their diameter: T1 (1 mm), T2 (>1 mm to 2 mm), T3 (>2 mm to 3 mm) and T4 (>3 mm), and each type was calculated as percent of the twenty openings inflicted on each fruit. The data were collected from 5 replicate fruit and are presented as average of each type ±SE. Natural openings on pricked fruit were ignored. Natural open lenticels on non-pricked fruit were determined as the percentage of SO_2-damaged lenticels per cm^2 area on the apple surface, containing damaged and undamaged lenticels, and results were averaged from 2 randomly picked apples from each of the 4 replicates (total 8 apples) with 2 observations per fruit ±SE. The SO_2 treatment was used to observe open lenticels on young developing fruit 45 d after anthesis, as well as on fruit at harvest and stored fruit (at 0 °C) after 2 and 4 months.

2.3. Microscopic examination

Lenticels at harvest, SO_2-detected lenticel damage on harvested apples, and damaged lenticels following storage (LB) were inspected by Leica MZFLIII stereomicroscope and scanning electron microscope (SEM). The microscopic images were digitally captured by a Nikon DS-Fi1 digital camera. For the scanning electron microscopy (SEM), samples were first dehydrated with ethanol, dried in K850 critical point dryer (Quorum Technology Ltd., UK), subsequently coated with gold-palladium alloy by SC7620 mini sputter coater (Quorum Technology Ltd., UK) and analyzed using benchtop SEM, JCM-600 (JEOL, Japan).

2.4. Assessment of natural lenticel breakdown after storage

Fruit harvested when commercially mature and stored in a cold room (0 °C, 82.5% RH), were analyzed for LB after 2 and 4 months storage. The experimental design consisted of 4 replicates of 40 fruit each, from which 2 fruits were sampled randomly at each examination time and exposed to SO_2 treatment to detect lenticel opening. The lenticel damage severity was calculated as the lenticel breakdown index (LBI) using the following formula:

$$LBI = \frac{LB_{\text{slight}} \times 1 + LB_{\text{intermediate}} \times 2 + LB_{\text{severe}} \times 4}{4}$$

where, LB_{slight}, $LB_{\text{intermediate}}$ or LB_{severe} represent the percentage of fruit with slight (1–3 damaged lenticels per fruit), intermediate (4–10) and severe (≥11) damage, respectively.

2.5. Statistical analysis

Statistical analysis of data was performed either by Student's *t*-test or by Tukey's HSD pairwise comparison tests at $P \leq 0.05$, as mentioned in each figure, using JMP 5.0.1a statistical software (SAS Institute Inc., N.C., USA).

3. Results

3.1. Sulfur dioxide (SO_2) treatment increases the size of artificial openings

Since, SO_2 has been known to cause damage to air-exposed tissue, effectiveness of the methodology using SO_2 treatment to detect exposed tissue to the air was evaluated by pricking the apple surface to cause air exposed perforation in the skin (Fig. 1). SO_2 treatment caused bleaching around these artificial openings and increased their dimensions, so that all T1 openings were enlarged, mainly to T3 and T4 types (Fig. 1). The percentage increment of T2, T3 and T4 was 17.4%, 186.36% and 106.25%, respectively compared to their respective types in non-exposed fruit (Fig. 1).

3.2. Response of lenticels to SO_2

The inspection of natural lenticels under microscope and by SEM revealed that these structures may be either open or closed.