

# Improvement of Apple Quality and Storability by a Combination of Heat Treatment and Controlled Atmosphere Storage

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**Abstract.** The effects of two nonchemical methods [controlled atmosphere (CA) storage and postharvest heating, alone or combined] on the quality (firmness, taste, color, and skin wax) and storability (losses resulting from bruising and fungal decay) of apples were investigated in a 3-year study. Fruits of two cultivars (cv. Aroma and cv. Ingrid Marie) were mechanically wounded on two opposing sides, inoculated with conidial suspensions of one of three pathogens [*Pezicula malicorticis* (bull's eye rot), *Penicillium expansum* (blue mould), and *Colletotrichum gloeosporioides* (bitter rot)], exposed to 40 °C for four different exposure periods (24, 48, 72, and 96 h), and stored either in air (21.0 kPa O<sub>2</sub> + 0.03 kPa CO<sub>2</sub>) or in CA storage (2.0 kPa O<sub>2</sub> + 2.0 kPa CO<sub>2</sub>) for 4 months. Effect of postharvest heating on bruise susceptibility of air- or CA-stored apples was also investigated. Cultivar Aroma apples generally showed higher bruise susceptibility than cv. Ingrid Marie. The sun-exposed side of apples was less sensitive to bruising than the shaded side and red phenotypes of these two cultivars also showed increased resistance to bruising as compared with standard phenotypes. Heat treatment and CA storage, either alone or in combination, decreased bruise occurrence in both cultivars. *P. malicorticis* was the more aggressive storage pathogen for both apple cultivars followed by *P. expansum* and *C. gloeosporioides*. The highest decay severity occurred in inoculated and nonheat-treated apples stored in air. Heat treatment, especially in combination with CA storage, showed an eradicated effect on the pathogens without any negative effects on apple quality. Heat treatment maintained flesh firmness during storage, reduced ethylene production, and caused clearly visible changes in epicuticular wax structure, resulting in a higher resistance to bruising or to natural and artificial infections with the pathogens. The effective exposure period could be reduced to 24 h, because a combination of heat treatment (at 40 °C for 24 h) and CA storage showed the best protective effect against bruising and fungal decay. This combined treatment decreased bull's eye rot by 86% and 60% and bitter rot by 73% and 65% in cv. Aroma and cv. Ingrid Marie, respectively, in comparison with untreated apples.

Most storage losses in the Swedish apple industry are caused by bruising and fungal decay. Harvesting and postharvest operations (especially grading, packing, and distribution) can cause a high degree of fruit bruising, resulting in a marked deterioration in quality (Ericsson, 1989). Although some bruising can be avoided by various orchard management practices (Tahir et al., 2005, 2007) and some storage systems can reduce fruit sensitivity to bruising, this problem is still difficult to

eliminate (Funt et al., 1999; Grajkowski et al., 2004; Zhang, 1994). Clear knowledge on cultivar susceptibility to bruising and on the ability of postharvest nonchemical treatments to reduce this susceptibility is needed by growers and fruit distribution organizations.

Infections by *Pezicula malicorticis* (bull's eye rot), *Penicillium expansum* (blue mould), and *Colletotrichum gloeosporioides* (bitter rot) are the main causes of decay during apple storage in Sweden (Tahir, 2006). Because pre- and postharvest application of fungicides is becoming banned in increasingly more countries, and totally prohibited in organic production (such as in Sweden), more environmental-friendly methods to protect apples against the more common fungal diseases should be developed.

Postharvest heat treatment has been used to reduce fungal rots in apples (Ferguson et al., 2000; Saftner et al., 2003; Sholberg et al., 2000). Decay caused by *P. expansum*

can be avoided by heating fruits at 38 °C for 4 d (Conway et al., 1994; Fallik et al., 1995; Leverentz et al., 2000). Contradictory results have been found for *C. gloeosporioides* with some studies reporting a positive effect of heat treatment (Conway et al., 2004; Rappel et al., 1991) and others no effect (Janisiewicz et al., 2003). The effects of heat treatment on *P. malicorticis* have not been carefully studied. Regarding apple quality, some authors report that heat treatment leads to faster apple softening and a deterioration in apple acidity and starch content (Conway et al., 2004; Leverentz et al., 2003), whereas others found heated apples sweeter and firmer than nonheated (Abbott et al., 2000; Saftner et al., 2003). Heat treatment is not yet widely used because of variability in effects resulting from infection time (Leverentz et al., 2000), temperature degree and exposure period (Lurie, 1998), and weak residual protection (Leverentz et al., 2003). However, it is important to resolve these practical difficulties with heat treatment and to evaluate the usefulness of heating in combination with other treatments such as biocontrol (Conway et al., 2004).

The mode of action of heat treatment is still unclear. Previous works reported a direct interaction with fungal germination and growth (Schirra et al., 2000) and/or physiological responses by apple tissue (Ben-Shalom et al., 1996). Postharvest heating is reported to affect fruit ripening by enhancing peel degreening (Klein and Lurie, 1992) and starch conversion (Conway et al., 2004) and by delaying fruit softening and volatile production (Fallik et al., 1995). Apple epicuticular wax is relatively smooth with numerous surface cracks arranged in interconnected networks. These networks disappear after heating as a result of recrystallization or melting of the wax platelets into the cracks (Lurie et al., 1996; Roy et al., 1994; Schirra et al., 2000). However, the relationship between structural changes in epicuticular wax and the resistance of heat-treated fruits to rots is still unclear.

This study investigated relationships between bruise occurrence and fungal decay in two apple cultivars with postharvest heating alone or in combination with controlled atmosphere (CA) storage. The aim was to determine the shortest effective exposure period required to significantly decrease bruising, and fungal rots resulting from *Pezicula malicorticis*, and *Colletotrichum gloeosporioides*, and maintain fruit quality and thereby develop a commercially attractive method to decrease storage losses.

## Materials and Methods

**Apples.** Two of the most commonly grown Swedish apple cultivars, cv. Aroma and cv. Ingrid Marie, were harvested from nonfungicide-treated trees at the experimental station orchard in Kivik, the Swedish University of Agricultural Sciences, during 2003–2006. Apples were picked at preclimacteric stage, when ethylene production

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was 4.0 pmol·kg<sup>-1</sup>·s<sup>-1</sup>, firmness was 79 to 82 N, and starch conversion was 4/10 for cv. Aroma and 3.0 pmol·kg<sup>-1</sup>·s<sup>-1</sup>, 86 to 88.0 N, and 3/10, respectively, for cv. Ingrid Marie (Tahir, 2006). After harvesting, apples were randomly divided into 65 lots per cultivar, 40 of which were used for pathogen experiments, 10 for quality control experiments, 10 for bruising experiments, and five for investigation of ethylene production and respiration rate. The first 60 lots comprised three replicate batches of 60 apples each, whereas the last five lots comprised three replicate batches of 150 apples each.

**Pathogens.** Of the 40 lots per cultivar, half were stored in air and the other half in CA storage after treatment. From each half:

1. Five lots were inoculated with conidial suspensions of *Pz. malicorticis*. One was then left unheated (control), whereas the other four lots were heat-treated at 40 ± 2 °C for 24, 48, 72, or 96 h.
2. Five lots were inoculated with conidial suspensions of *P. expansum* and then treated as in (1).
3. Five lots were inoculated with conidial suspensions of *C. gloeosporioides*, and then treated as in (1).
4. Five lots were used to investigate the natural infection (without inoculation). One was left unheated (control), whereas the other four lots were heat-treated at 40 ± 2 °C for 24, 48, 72, or 96 h.

The three pathogens used were isolated from naturally infected apples showing typical symptoms of the diseases, maintained on petri dishes with potato dextrose agar, and stored separately as pure cultures at 4 °C. Pathogen virulence over time was confirmed by periodic transfers through apples. For the present experiments, spores of the three pathogens were removed from the surface of 10-d-old cultures and suspended in 5 mL sterile distilled water containing 0.05% (v/v) Tween 80. The suspensions were filtered through four layers of sterile cheesecloth to remove any adhering mycelia, and spore concentrations were adjusted to 1 × 10<sup>5</sup> conidia/mL (by hemacytometer). The apples used were washed for 2 min with water to remove natural occurring fungi and wounded twice on both sides to a depth of 2 mm with a nail (4 mm diameter); one wound was placed 10 mm above the equator and the other 10 mm below and inoculated by pipetting 20 µL of freshly prepared conidial pathogen suspension into each of the wound sites.

**Bruising.** Of the 10 lots per cultivar used for the bruising experiments, half were stored in air and the other in CA storage. From each half, one lot was stored within 6 h of harvesting (control), and the other four lots were heat-treated at 40 ± 2 °C for 24, 48, 72, or 96 h before storage. After 4 months of storage, apples were tested for bruise susceptibility by dropping them from 30 cm through vertical hollow polyvinyl chloride pipe onto a wooden table. Bruised fruits were watched

and examined after 12 h (when bruised area totally browned at room temperature). They were cut longitudinally; bruise depth (h), diameter (d), apple diameter (D) and altitude (x) were measured; and brown tissue was excised and weighed. Bruise susceptibility was estimated as: bruise volume (BV) =  $6^{-1} \pi h d^2$  (Chen and Sun, 1981). To evaluate the relationship between coloration and bruise susceptibility, an additional lot of 150 apples per cultivar (cvs. Aroma and Ingrid Marie and their red phenotypes cv. Amorosa and cv. Karin Schneider) were picked at commercial harvesting dates, divided equally in three replicates each, and bruised within 6 h after picking by dropping them on their exposed or shaded side as described previously. Apple coloration of each side was measured as described subsequently.

**Apple quality.** Of the 10 lots per cultivar used for fruit quality experiments, half were stored in air and the other half in CA storage. One lot from each half was stored within 6 h of harvesting as control and the other four lots were heat-treated at 40 ± 2 °C for 24, 48, 72, or 96 h. After storage, apple quality was monitored using 45 apples. Apple coloration was measured as hue angle (h°) using a Chromameter CR200 with a 8-mm diameter window (Minolta Ltd., Osaka, Japan). Measurements were conducted on three widely spaced spots at the apple equator and expressed as h°, in which 0° = red–purple, 90° = yellow, 180° = bluish green, and 270° = blue (McGuire, 1992). Apple firmness was determined by penetrometer (Model FT-327; Effigy, Alfonsine, Italy; plunger diameter 11.1 mm, depth 7.9 mm) on opposite sides of each apple and the results expressed as force (N) required for penetration. Soluble solids content (SSC) in apple juice was measured by a refractometer (Atago, Tokyo, Japan) and the results expressed as a percentage. Titratable acidity was analyzed by titration of apple juice as malic acid with 0.05N NaOH to pH 8.1 (Radiometer PHM64, Copenhagen, Denmark). Storage rot (natural infection) was determined by visual examination according to Rein (1996). Decay severity resulting from artificial inoculation was measured as surface decay lesion area. Skin of heated and unheated apples was examined under a scanning electronic microscope (LEO 435VP, Cambridge, UK) with secondary electron detector at 2 kV low voltage and on magnification 1000×. Two apples per replicate batch and lot were assessed. Equatorial peel (4 to 5 mm<sup>2</sup>) from either the sun-exposed or shaded side of apples was freshly mounted on double-sided tape and on specimen stubs. Areas of wax at least 5 µm thick were chosen.

**Respiration activity and ethylene production.** Of the five lots per cultivar used for respiration rate and ethylene production determination, one was left untreated (control), whereas the other four lots were heat-treated on 40 ± 2 °C for 24, 48, 72, or 96 h. All lots were stored in air (21.0 kPa O<sub>2</sub> + 0.03 kPa CO<sub>2</sub>, 2 ± 0.5 °C and 90% relative humidity). Once a week, six apples from each lot were transferred to 0.5-L sealed glass jars (three

replicate jars per treatment) for measurement of apple respiration rate and ethylene production. The jars were hermetically sealed with rubber stoppers for 2 h at 20 °C before measurements. One milliliter of holder atmosphere was withdrawn by gas syringe and the ethylene quantified by gas chromatography, Agilent 6850 (column Agilent 19095P-Q04E Hp-Plot Q 30 m × 530 µm × 40 µm). For respiration rate measurements (CO<sub>2</sub>), one additional milliliter was analyzed by gas chromatography, Varian Inc. 3700 (two-column Hayesep Q1/4" × 8" and Molsict 5, 3/8" × 3").

**Heat treatment.** At harvest, the field temperature of cv. Aroma and cv. Ingrid Marie was 16 and 14 °C, respectively. Within 6 h of inoculation, apples were placed in separate new plastic boxes, covered with polyethylene to provide water-saturated conditions and to protect the apple from heat damage (Miller et al., 1990), and kept in thermostatically controlled (± 2 °C) small chambers (4.5 m<sup>3</sup>) at 40 °C for 24, 48, 72, or 96 h. Relative humidity (RH) was always more than 80%. The heated apples were allowed to equilibrate to room temperature for 12 h before being transferred to air or CA storage.

**Storage and shelf life.** Apples were stored either in air (21.0 kPa O<sub>2</sub> + 0.03 kPa CO<sub>2</sub>, 2 ± 0.5 °C and 90% RH) or in CA (2.0 kPa O<sub>2</sub> + 2.0 kPa CO<sub>2</sub>, 2 °C and 90% RH) (Haffner, 1993). CA storage conditions with computer-controlled gas composition were established in 350-L chambers (Nino-laboratory, Onsala, Sweden) 3 d before placing the apples in the chambers. At the end of storage, all apples were transferred to a plastic chamber (18 ± 2 °C and 80% RH) for 1 week before quality and natural and artificial infections with *Pz. malicorticis*, *P. expansum*, and *C. gloeosporioides* were evaluated. Changes in quality parameters were also estimated.

**Statistical analyses.** The experiment was carried out over three seasons. Three variables were controlled: two storage methods, five exposure periods, and three seasons. Decay incidence was defined as the percentage of natural occurring fungal infection and decay severity was measured as surface decay lesion area caused by artificial infection. Data were subjected to arcsine square root transformation before analysis. Data were subjected to analyses of variance using the general linear model to determine main effects and interactions (SAS Inc., Cary, NC). Least significant difference values (0.05) were calculated for comparison of means. Regression analyses and Pearson correlation were carried out (using SAS) to quantify the relationships among bruising occurrence, heat exposure time, and skin color.

## Results

**Apple quality.** Fruit firmness at the end of shelf life was affected by cultivar, storage method, season, and treatment (Table 1; *P* < 0.05). The highest loss in firmness was found for control apples of either cultivar, whereas

Table 1. Reduction of flesh firmness of heated and non heated apple fruits during storage and shelf life.

Control time	Exposure period (h)	Cultivar Aroma <sup>z</sup>			Cultivar Ingrid Marie <sup>z</sup>		
		2003	2004	2005	2003	2004	2005
At harvest		80.0	82.0	79.0	88.0	86.0	87.0
After storage in air:	(0) Control	48.3 e	52.3 d	50.0 e	55.0 f	59.7 f	57.3 c
21.0 kPa O <sub>2</sub> +	24	54.0 d	53.7 cd	54.0 d	61.7 e	61.7 f	64.3 b
0.03 kPa CO <sub>2</sub>	48	58.7 bcd	57.0 b	59.0 bc	66.7 cd	65.0 cde	65.0 b
	72	57.0 cd	54.7 cd	57.3 c	65.3 cde	62.0 ef	65.3 b
	96	56.0 cd	56.0 bc	56.7 cd	63.7 de	63.7 def	65.0 b
After storage in CA:	(0)	56.0 cd	56.2 bc	58.0 c	57.7 f	68.7 abc	64.7 b
2.0 kPa O <sub>2</sub> +	24	64.0 a	62.0 a <sup>y</sup>	64.3 a <sup>y</sup>	70.0 b	70.3 ab	65.4 b
2.0 kPa CO <sub>2</sub>	48	66.7 a <sup>y</sup>	58.3 b	61.0 b	74.7 a <sup>y</sup>	72.3 a <sup>y</sup>	69.7 a <sup>y</sup>
	72	59.7 bc	58.7 b	59.3 b	66.0 cd	67.0 bcd	68.3 ab
	96	60.3 b	61.3 a	59.7 b	69.0 bc	66.3 cd	65.3 b

<sup>z</sup>Values are means of three replicates of 60 fruits each. n = 1350. Significant interaction was found between seasons and storage method-treatment at  $P < 0.05$ .

<sup>y</sup>Means followed by different letters within the same column are significantly different at least significant difference = 0.05.

CA = controlled atmosphere.

the loss was significantly lower for apples heated at 40 °C for a minimum of 48 h (or 24 h in 2003 for cv. Aroma and in 2005 for cv. Ingrid Marie) and for apples stored in CA (Table 1). Combined heat treatment (for 24 h for cv. Aroma and 48h for cv. Ingrid Marie) and CA storage maintained better firmness, showing 26%, 12%, and 10% higher firmness in comparison with control, CA storage, and heat treatment (at 40 °C for 24 or 48 h), respectively (Table 1). Extending the heat exposure period more than 24 h for cv. Aroma and 48 h for cv. Ingrid Marie did not improve the effect of heat treatment on apple firmness, and instead had a negative influence for cv. Aroma in 2003 and 2005 (Table 1). No relationships were found between SSC or acidity and the heat treatments or CA storage in either cultivar, whereas heating apples at 40 °C for 24 h before CA storage improved the maintenance of the taste by 15% (higher acidity:SSC ratio) in comparison with other treatments (Table 2;  $P < 0.05$ ). Heat-treated apples had better coloration (lower  $h^{\circ}$  value) after shelf life compared with untreated fruit (Table 2). No interactions between treatment effect and season or between exposure period and col-

oration were found. Nonheated apples (control) showed higher ethylene production than those heated at 40 °C. This difference was first detectable in cv. Aroma after 5 weeks and in cv. Ingrid Marie after 6 weeks of air storage (Table 3;  $P < 0.05$ ). Ethylene production was inversely related to exposure period. This effect became gradually visible until the last week in which the lowest ethylene production was found in apples heated at 40 °C for 96 h followed by heating for 72, 48, and 24 h (Table 3). Heat-treated apples showed higher respiration rate than nonheated during the first week in air storage, but this changed later (Weeks 5 to 7 in cv. Aroma and Weeks 3 to 7 in cv. Ingrid Marie) when nonheated apples showed a higher respiration rate than heat-treated apples. No visible influence of exposure time was observed in the beginning, whereas after 2 months of storage, heat-treated apples for 24 to 48 h showed higher respiration rate than heat-treated apples for 96 h (Table 4). The wax layer on the sun-exposed side of cv. Aroma apples usually had clumps (Fig. 1A1), whereas the wax layer on the shaded side often had nail-like crystals (Fig. 1A2). Cracks and fungal hyphae occurred on both

sides, but particularly on the shaded side (Fig. 1A3). Wax layers in cv. Ingrid Marie were heavier, thicker, and less homogenous than those in cv. Aroma. Wax with nail-like crystals, microcracks, and fungal hyphae were found on both sun-exposed (Fig. 2A1) and shaded sides (Fig. 2A2).

**Bruising.** Bruising after storage was affected by cultivar, season, and fruit side. Cultivar Aroma and cv. Ingrid Marie apples were significantly ( $P < 0.05$ ) more sensitive to bruising than the respective red phenotype cv. Amorosa (by 15% on the shaded side and 12% on the sun-exposed side) and cv. Karin Schneider (by 14% on the shaded side and 10% on the sun-exposed side during only two seasons) (Table 5). The shaded side of fruit was more sensitive (larger BV) to bruising than the sun-exposed side (Table 5). Generally, a positive Pearson correlation was found between  $h^{\circ}$  value and bruise occurrence with fruit with high  $h^{\circ}$  value (poor red color) showing larger BV than fruit with low  $h^{\circ}$  value (Table 5). Heating apples decreased bruise susceptibility (lower BV) in both cultivars (Table 6). A positive correlation was found between heat exposure period and BV. The shortest effective exposure period (at 40 °C) was 24 h in cv. Aroma, decreasing BV by 58%, and 48 h in cv. Ingrid Marie, decreasing BV by 43%, compared with unheated fruits (Table 6). A combination of postharvest heat treatment (at 40 °C for 24 h) and CA storage increased the positive effect of each separate treatment on bruise susceptibility of the two cultivars. This combined treatment reduced BV by 54% and 40% compared with only CA storage and by 23% and 35% compared with only heat treatment (at 40 °C for 24 h as shortest effective period) in cv. Aroma and cv. Ingrid Marie apples, respectively (Table 6). No significant interactions with season were noted.

**Fungal decay resulting from natural infection.** Natural decay incidence was affected by cultivar, decay-causing agent, season, storage method, and treatment. Cultivar Ingrid Marie apples showed more resistance to *Pz. malicorticis* than cv. Aroma apples (by 30%), whereas cv. Aroma was always more sensitive to *P. expansum* than cv. Ingrid Marie (by 40%). Resistance to *C. gloeosporioides* varied between the seasons (data not shown). CA storage decreased decay resulting from the three pathogens by 50% compared with the air stored cv. Aroma apples (Table 7) and by 50%, 38%, and 27% resulting from *Pz. malicorticis*, *C. gloeosporioides*, and *P. expansum*, respectively, compared with the air stored cv. Ingrid Marie apples (Table 7). Heat treatment at 40 °C for 24 h reduced storage decay in cv. Aroma as a result of each of the three pathogens by 28% in air and 62% in CA (Table 7), whereas decay resulting from *Pz. malicorticis* and *C. gloeosporioides* in cv. Ingrid Marie apples was also decreased by 25% and 33% in fruits stored in air and by 50% and 38% in fruits stored in CA, respectively, in comparison with control. This positive influence on decay

Table 2. Changes in skin color and fruit taste of heated and nonheated apple fruits during storage and shelf life.

Control time	Exposure period (h)	Acidity/SSC (%) <sup>z</sup>		Color ( $h^{\circ}$ ) value <sup>z</sup>	
		Cultivar Aroma	Cultivar Ingrid Marie	Cultivar Aroma	Cultivar Ingrid Marie
At harvest		9.0	8.3	91.3	35.6
After storage in air:	(0) Control	4.1 e	4.2 b	80.1 a <sup>y</sup>	33.2 a <sup>y</sup>
21.0 kPa O <sub>2</sub> +	24	4.4 cde	4.4 ab	67.4 b	28.3 b
0.03 kPa CO <sub>2</sub>	48	4.5 bcd	4.6 ab	67.0 b	28.9 b
	72	4.4 cde	4.7 ab	66.8 b	29.1 b
	96	4.5 bcd	4.5 ab	66.8 b	28.2 b
After storage in CA:	(0)	4.2 de	4.2 b	86.8 a	35.1 a
2.0 kPa O <sub>2</sub> +	24	4.9 a <sup>y</sup>	4.8 a	72.1 b	30.4 b
2.0 kPa CO <sub>2</sub>	48	4.8 ab	4.9 a <sup>y</sup>	71.0 b	28.9 b
	72	4.7 abc	4.9 a	71.0 b	30.7 b
	96	4.6 abc	4.6 ab	71.6 b	29.6 b

<sup>z</sup>Values are transformed means of three seasons, three replicates of 60 fruits each. n = 450, because no significant interaction was noted between seasons treatments at  $P < 0.05$ . Significant interaction was found only between storage methods and exposure periods.

<sup>y</sup>Means followed by different letters within the same column are significantly different at least significant difference = 0.05.

SSC = soluble solids content; CA = controlled atmosphere.



Table 3. Ethylene production in heated and non heated apple fruits during storage in air.

Cultivar	Heating at 40 °C for:	Ethylene production (pmol·kg <sup>-1</sup> ·s <sup>-1</sup> ) during storage in air (weeks) <sup>z</sup>			
		Week 5	Week 6	Week 7	Week 8
Aroma	0 h Control	147.2 a <sup>y</sup>	490.5 a	790.0 a	1,222.8 a
	24 h	62.8 b	182.8 b	298.5 b	863.8 b
	48 h	101.9 ab	139.2 bc	223.0 bc	725.2 c
	72 h	100.7 ab	98.4 c	178.3 cd	630.7 d
	96 h	97.9 ab	81.3 c	135.5 d	505.2 e
Ingrid Marie	0 h Control	111.0 A <sup>y</sup>	444.2 A	1,018.5 A	1,376.9 A
	24 h	89.4 A	228.8 B	334.2 B	722.6 B
	48 h	85.5 A	187.7 BC	230.4 C	626.9 C
	72 h	84.4 A	156.2 BC	176.6 CD	505.4 D
	96 h	82.2 A	107.5 C	134.3 D	403.3 E

<sup>z</sup>Values are means of two seasons, three replicate of one fruit each. n = 240, because no significant interaction was noted between seasons-treatments at  $P < 0.05$ . Significant interaction was only found between exposure period and storage week at  $P < 0.05$ , starting in Week 6.

<sup>y</sup>Means followed by different letters within the same column are significantly different at least significant difference = 0.05; lower case letter refers to 'Aroma' and upper case letter refers to 'Ingrid Marie'.

Table 4. Respiration rate (CO<sub>2</sub> production) in heated and nonheated apple fruits during storage in air.

Cultivar	Heating at 40 °C for:	CO <sub>2</sub> production (pmol·kg <sup>-1</sup> ·s <sup>-1</sup> ) during storage in air (weeks) <sup>z</sup>							
		1	2	3	4	5	6	7	8
Aroma	0 h Control	42.7 b <sup>y</sup>	54.7 a	72.2 a	86.7 a	115.4 a	129.7 a	137.8 a	161.9 a
	24 h	62.3 a	51.8 a	62.3 a	72.3 a	65.9 b	76.8 b	87.3 b	117.4 b
	48 h	60.4 a	54.4 a	67.7 a	73.6 a	70.7 b	81.3 b	84.3 b	107.5 b
	72 h	61.0 a	52.3 a	68.8 a	68.0 a	63.2 b	74.7 b	80.1 b	99.2 bc
	96 h	63.4 a	49.2 a	65.3 a	67.6 a	67.1 b	70.3 b	78.6 b	77.9 c
Ingrid Marie	0 h Control	48.0 B	61.5 A	81.2 A	97.4 A	110.5 A	114.8 A	143.7 A	170.7 A
	24 h	64.3 A	58.2 A	63.3 B	81.3 B	74.0 B	73.2 B	98.1 B	126.3 B
	48 h	65.2 A	61.1 A	64.7 B	82.7 AB	79.5 B	74.5 B	94.7 B	120.8 B
	72 h	65.8 A	58.8 A	66.8 B	76.4 B	78.5 B	76.3 B	90.0 B	111.5 BC
	96 h	65.8 A	55.3 A	64.0 B	82.5 AB	86.2 B	79.0 B	88.3 B	102.5 C

<sup>z</sup>Values are means of two seasons, three replicate of one fruit each. n = 240, because no significant interaction was noted between seasons treatments at  $P < 0.05$ . Significant interaction was only found between exposure period and storage week at  $P < 0.05$ .

<sup>y</sup>Means followed by different letters within the same column are significantly different at least significant difference = 0.05; lower case letter refers to cv. Aroma and upper case letter refers to cv. Ingrid Marie.

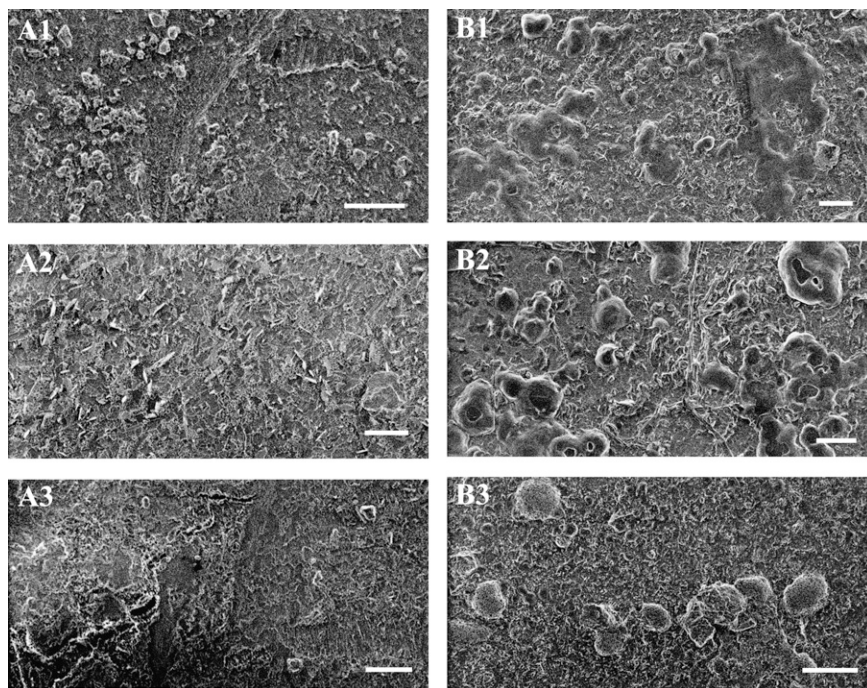


Fig. 1. Epicuticular wax in cv. Aroma apples (A) in untreated and (B) in heat-treated apples at 40 °C for 24 h. Scale bars = 5 μm, magnification 1000x; Leo. 435 up. (A1) Wax clumps on sun-exposed side. (A2) Nail-like wax crystals on shaded side. (A3) Crack in wax layer, shaded side. (B1) Wax clumps fusing as a result of heat treatment. (B2) Fungal hyphae captured in melted wax. (B3) Wax layer on shaded side less influenced by heat treatment.

caused by *P. expansum* was shown only when heat treatment was combined with CA storage (Table 7).

**Fungal decay resulting from artificial inoculation.** Decay resulting from artificial inoculation was affected by cultivar, decay-causing agent, storage method, and treatment. In both cultivars, *Pz. malicorticis* caused the largest lesion area (13.0 to 17.0 cm<sup>2</sup>) followed by *P. expansum* (8.2 to 14.0 cm<sup>2</sup>), whereas *C. gloeosporioides* gave the smallest lesions (4.0 to 4.8 cm<sup>2</sup>). Heating apples at 40 °C for 24 h in air gave 50%, 63%, and 56% smaller lesion area in cv. Aroma and 48%, 56%, and 32% smaller lesion area in cv. Ingrid Marie resulting from inoculation with *Pz. malicorticis*, *C. gloeosporioides*, and *P. expansum*, respectively, compared with the nonheat-treated apples (Table 8). The shortest protective exposure period was 24 h, because no further beneficial effect with longer exposure periods was observed (Table 8). CA storage decreased decay severity resulting from *Pz. malicorticis*, *C. gloeosporioides*, and *P. expansum* by 53%, 52%, and 50% in cv. Aroma and by 40%, 16%, and 50% in cv. Ingrid Marie, respectively, in comparison with the air-stored apples (Table 8). Combined heat treatment (40 °C for 24 h) and CA storage improved resistance to *Pz. malicorticis*, *C. gloeosporioides*, and *P. expansum* further and resulted in lower decay severity compared with only CA-stored apples (by 60%, 70%, and 50%, respectively, in cv. Aroma apples and by 60%, 57%, and 40% in cv. Ingrid Marie) (Table 8).

**Changes in skin wax.** Heat treatment caused important changes in epicuticular wax. After heating of cv. Aroma apples at 40 °C for 24 h, wax clumps started to fuse together to discernibly continuous parallel layers (Figs. 1B1 and 2B1) with occasional remains of fungal hyphae captured in the wax (Figs. 1B3 and 2B3). However, the degree of wax fusion varied even within the same apple. The nail-like wax crystals held their form longer than the wax clumps (Fig. 2B2). Heating had less effect on the wax structure of cv. Ingrid Marie apples. However, micro-crack structure was altered by heating and filled with melted wax (Figs. 1B1 and 2B1). Fungal hyphae were also captured in the wax (Figs. 1B3 and 2B3).

## Discussion

Bruising was not totally prevented by CA storage, but bruise volume was decreased by combining CA storage with heat treatment at 40 °C for 24 h, to commercially acceptable levels in both cultivars, because apples with bruised area smaller than 1 cm<sup>3</sup> in volume were accepted by consumers (Ericsson and Tahir, 1996). The inhibitory effect of heat treatment on ethylene production and uronic acid degradation (Klein et al., 1990) and synthesis of cell wall hydrolytic enzymes such as polygalacturonase (Lurie et al., 1996), resulting in better firmness might cause higher ability to withstand impact injury. A clear correlation was found between

apple red color and resistance to bruising, because shaded sides were more sensitive to impacts than sun-exposed sides. Also, cvs. Aroma and Ingrid Marie had higher bruise susceptibility than their red phenotypes cvs.

Amorosa and Karin Schneider. The sun-exposed side of apples contained thicker and more numerous wax clumps than the shaded side. Heat treatment is known to melt skin wax and change its structure (Roy et al.,

1994), perhaps providing some cushioning and decreasing the impact pressure. Cultivar Ingrid Marie apples, which had thicker, nail-like, and heavier wax, also had higher resistance to bruising than cv. Aroma.

*C. gloeosporioides* did not cause severe decay in either cultivar, whereas the dominant storage pathogen was *Pz. maliortidis* followed by *P. expansum*. Both of these fungal species were the more aggressive pathogens and not only *P. expansum* as previously suggested (Conway et al., 2004; Janisiewicz et al., 2003). All three pathogens were sensitive to heat treatment (at 40 °C) reducing natural fungal infection or decay resulting from artificial inoculation of apples. The effectiveness of heat treatment depended on cultivar and decay-causing agent, confirming previous studies (Barkai-Golan and Philips, 1991; Spadaro et al., 2004), although the present study did not indicate any positive effect of extending heat exposure period on the reduction of the decay.

Shortening the exposure period of heat treatment from 96 to 24 h did not cause any negative change in the method effectiveness. Heating apples for longer than 48 h increased

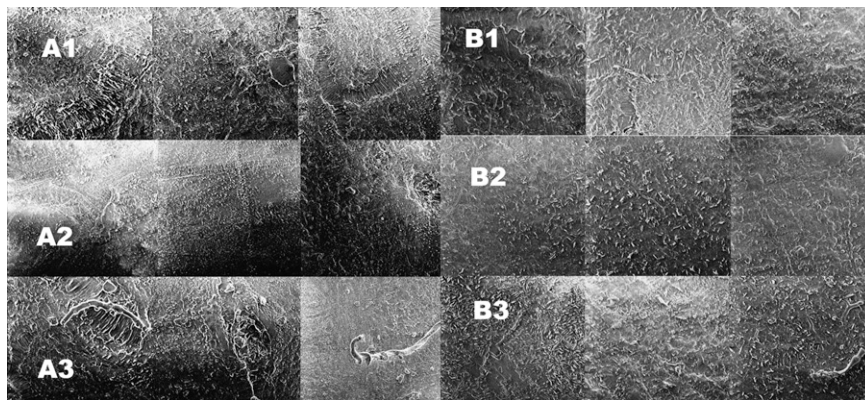


Fig. 2. Epicuticular wax in cv. Ingrid Marie apples (A) in untreated and (B) in heat-treated apples at 40 °C for 24 h. Scale bars = 5 µm, magnification 1000×; Leo. 435 up. (A1) Layers of nail-like wax crystals on shaded side. (A2) Wax clumps and nail-like crystals on exposed side. (A3) Crack and hyphae in the wax layer. (B1) Wax clumps fusing very slowly resulting from heat treatment. (B2) Melted wax filling cracks. (B3) Fungal hyphae captured in melted wax.

Table 5. Relationships between apple color and bruise occurrence.

Cultivar	Group	Bruised side	2003			2004			2005		
			BV (%) <sup>z</sup>	Red color (h°) <sup>y</sup>	Pearson correlation	BV (%) <sup>z</sup>	Red color (h°) <sup>y</sup>	Pearson correlation	BV (%) <sup>z</sup>	Red color (h°) <sup>y</sup>	Pearson correlation
Aroma	Standard	Shaded	3.2 a <sup>x</sup>	120.9 a	0.99***	3.0 a	114.5 a	0.84***	2.9 a	128.0 a	0.92***
		Exposed	2.6 b	82.3 b	0.40*	2.8 b	76.3 b	0.88***	2.7 b	83.3 b	0.97***
	Red	Shaded	2.7 b	59.9 c	0.85***	2.6 c	54.7 c	0.99***	2.5 c	49.1 c	0.98***
		Exposed	2.4 c	52.1 d	0.21*	2.4 d	39.1 d	0.74**	2.5 c	46.7 c	0.62**
Ingrid Marie	Standard	Shaded	2.5 a <sup>x</sup>	55.0 a	0.99***	2.5 a	54.8 a	0.99***	2.4 a	37.0 a	0.54*
		Exposed	2.3 b	28.3 b	0.99***	2.1 bc	30.7 b	0.94***	2.2 b	24.4 b	0.81***
	Red	Shaded	2.3 b	27.3 b	0.89***	2.2 b	21.5 c	0.45	2.0 c	20.7 c	0.24
		Exposed	2.1 c	20.3 c	0.62**	2.0 c	19.3 c	0.57*	1.9 c	20.0 c	0.96***

Fruits were bruised within 6 h after harvesting.

<sup>z</sup>BV = bruise volume percentage (bruise volume \* fruit volume<sup>-1</sup>).

<sup>y</sup>Lower h° means better red coloration. Values are transformed means of three replicates of 50 fruits each. n = 150.

\*Means followed by different letters within the same column and cultivar are significantly different at least significant difference = 0.05.

\*, \*\*, \*\*\* = Significant at *P* < 0.05, 0.01, and 0.001, respectively. Interactions cultivar-fruit side, cultivar-season, fruit side-season were significant at *P* < 0.05.

“K.S.” = cv. Karin Schneider; Red Aroma = cv. Amorosa.

Table 6. Postharvest heating at 40 °C increases apple resistance to bruising (average of the means 2003–2005).

Storage methods	Treatment period (h)	Cultivar Aroma BV (%) <sup>z</sup>	Pearson correlation (r) between bruising and heat-exposure periods	Cultivar Ingrid Marie BV (%) <sup>z</sup>	Pearson correlation (r) between bruising and heat-exposure periods
Air: 21.0 KPa O <sub>2</sub> and 0.03 KPa CO <sub>2</sub>	0 h Control	3.1 a <sup>y</sup>		2.3 a <sup>y</sup>	
	24	1.3 g		1.7 bc	
	48	1.6 ef	0.85**	1.3 de	0.57*
	72	1.9 cd		1.5 cd	
	96	2.0 bc		2.1 ab	
CA storage: 2.0 KPa O <sub>2</sub> and 2.0 KPa CO <sub>2</sub>	0 h	2.2 b		1.9 b	
	24	0.9 h		1.2 ef	
	48	1.4 fg	0.84**	0.8 g	0.37
	72	1.5 efg		1.1 ef	
	96	1.7 de		1.5 cd	

<sup>z</sup>Fruits were bruised after storage and all values are means of the bruise volume on both fruit sides. BV values: bruise volume percentage (bruise volume \* fruit volume<sup>-1</sup>) are transformed means of three seasons (2003–2005) and three replicates of 540 bruised areas.

\*Means followed by different letters within a column for bruise volume are significantly different at least significant difference = 0.05. Mean squares from GLM analyses of bruise volume: between exposure periods significant at *P* < 0.05, interaction exposure periods–storage method was significant at *P* < 0.05, and between period–season was not significant at *P* < 0.05.

\*, \*\*, \*\*\* = Significant at *P* < 0.5, 0.01, and 0.001, respectively.

CA = controlled atmosphere.



Table 7. Natural fungal decay in heated and nonheated apples after 4 months of storage and 1-week shelf life.

Aroma										
Storage methods	Exposure periods (h)	<i>Pezizula malicorticis</i> <sup>z</sup> (%)			<i>Colletotrichum gloeosporioides</i> <sup>z</sup> (%)			<i>Penicillium expansum</i> <sup>z</sup> (%)		
		2003	2004	2005	2003	2004	2005	2003	2004	2005
Air: 2.0 kPa O <sub>2</sub> + 0.03KPa CO <sub>2</sub>	(0) Control	12.7 a <sup>y</sup>	9.1 a <sup>y</sup>	10.0 a <sup>y</sup>	5.6 a <sup>y</sup>	4.9 a <sup>y</sup>	3.7 a <sup>y</sup>	7.9 a <sup>y</sup>	6.1 a <sup>y</sup>	6.0 a <sup>y</sup>
	24	10.0 b	7.1 b	8.3 bc	4.4 b	4.0 b	2.8 ab	6.2 b	4.8 b	4.8 b
	48	9.5 b	6.2 bc	8.5 b	4.0 bc	3.8 b	2.9 ab	5.9 b	4.4 bc	4.9 b
	72	7.4 c	5.5 cd	7.2 bc	3.3 cd	3.3 b	2.8 ab	4.6 c	3.8 de	4.3 b
	96	8.3 bc	6.3 bc	7.0 c	3.8 bc	3.5 b	2.3 bc	5.2 c	4.2 cd	4.0 bc
CA: 2.0 kPa O <sub>2</sub> + 2.0 KPa CO <sub>2</sub>	(0)	6.4 d	3.9 e	5.5 d	2.6 de	2.4 c	2.0 bc	3.9 d	2.8 f	3.2 cd
	24	2.7 f	1.9 f	1.3 g	2.1 e	1.9 cd	1.6 bc	2.1 f	1.6 g	1.3 f
	48	4.2 ef	3.9 e	3.5 ef	1.3 f	1.4 d	1.0 c	2.4 f	2.3 f	1.9 ef
	72	3.8 ef	4.2 de	2.8 f	2.1 e	1.8 cd	1.7 bc	2.5 f	2.6 f	1.9 ef
	96	4.7 e	5.5 c	4.2 de	2.6 de	4.9 a	1.0 c	3.2 e	3.4 e	2.3 de
Ingrid Marie										
Storage methods	Exposure periods (h)	<i>Pezizula malicorticis</i> <sup>z</sup> (%)			<i>Colletotrichum gloeosporioides</i> <sup>z</sup> (%)			<i>Penicillium expansum</i> <sup>z</sup> (%)		
		2003	2004	2005	2003	2004	2005	2003	2004	2005
Air: 21.0 kPa O <sub>2</sub> + 0.03 KPa CO <sub>2</sub>	(0) Control	10.7 a <sup>y</sup>	5.5 a	6.3 a	3.5 a	4.4 a	5.2 a	5.5 a	3.3 c	3.6 a
	24	9.2 b	6.1 a	4.6 b	2.4 b	2.8 bc	3.1 b	6.4 a	4.8 a	3.6 a
	48	6.6 c	5.4 a	3.8 bc	2.1 bc	2.5 bc	3.8 b	4.8 ab	4.1 b	2.9 ab
	72	6.9 c	5.5 a	4.4 b	2.3 bc	2.5 bc	4.1 ab	4.8 ab	4.1 b	3.1 ab
	96	7.5 c	4.2 b	3.5 bcd	2.1 bc	2.9 bc	4.1 ab	4.7 ab	3.3 c	2.7 ab
CA: 2.0 kPa O <sub>2</sub> + 2.0 KPa CO <sub>2</sub>	(0)	5.0 d	3.3 cd	2.7 cde	2.1 bc	2.4 bc	3.6 b	3.8 bc	2.9 c	2.2 bc
	24	1.9 f	2.8 de	1.5 e	1.5 c	1.5 c	3.3 b	2.0 d	2.3 d	1.5 c
	48	2.9 ef	1.5 f	1.7 e	1.6 c	1.9 c	3.7 b	2.2 d	1.5 e	1.4 c
	72	3.1 ef	2.3 ef	2.6 de	1.5 c	3.3 ab	5.2 a	1.9 d	1.6 e	1.6 c
	96	4.3 de	3.3 cd	2.2 e	1.5 c	4.2 a	3.3 b	2.8 cd	2.2 d	1.7 c

<sup>z</sup>Values are transformed means of three replicates of 60 fruits each. n = 1800. Interactions cultivar-agent, cultivar-season, agent-season, cultivar-storage method, and storage method-agent were significant at  $P < 0.05$ .

<sup>y</sup>Means followed by different letters within the same column are significantly different at least significant difference = 0.05.

CA = controlled atmosphere.

Table 8. Improvement of apple resistance to fungal decay [as lesion area (cm<sup>2</sup>)] by postharvest heating and controlled atmosphere (CA) storage (means 2003–2005).

Storage methods	Treatment period (h)	Cultivar Aroma <sup>z</sup>			Cultivar Ingrid Marie <sup>z</sup>		
		<i>Penicillium expansum</i>	<i>Pezizula malicorticis</i>	<i>Colletotrichum gloeosporioides</i>	<i>Penicillium expansum</i>	<i>Pezizula malicorticis</i>	<i>Colletotrichum gloeosporioides</i>
Air: 21.0 kPa O <sub>2</sub> + 0.03 kPa CO <sub>2</sub>	(0) Control	8.2 a <sup>y</sup>	17.1 a	4.8 a	14.0 a	13.0 a	4.0 a
	24	4.2 bc	6.2 cd	2.1 cd	7.3 c	5.7 cd	2.7 b
	48	4.2 bc	7.5 bc	2.2 cd	6.6 c	5.1 e	2.0 c
	72	4.5 b	8.4 b	2.7 bc	7.2 bc	6.0 c	2.3 bc
	96	4.5 b	7.7 bc	3.0 b	8.3 b	6.0 c	2.4 bc
CA: 2.0 kPa O <sub>2</sub> + 2.0 kPa CO <sub>2</sub>	(0)	3.8 bc	8.1 b	2.4 bc	8.4 b	10.9 b	2.0 c
	24	1.5 e	2.4 f	1.3 e	3.5 e	4.9 e	1.4 d
	48	2.2 d	3.4 ef	1.0 e	3.0 e	4.3 f	1.0 d
	72	2.2 d	4.5 de	1.6 de	3.5 e	5.4 de	1.0 d
	96	3.2 c	4.9 de	1.6 de	5.4 d	5.9 cd	1.2 d

<sup>z</sup>Mean squares from GLM analyses of lesion area showed no significant interactions exposure periods-seasons. Values are transformed means of three seasons (2003–2005) and three replicates of 540 inoculation areas.

<sup>y</sup>Means followed by different letters within the same columns for either pathogen are significantly different at least significant difference = 0.05.

skin damage, particularly in stored cv. Ingrid Marie, possibly because of stomata destroyed at higher heat becoming sites of subsequent decay (data not shown). The positive effects of combined heat treatment and CA storage on bruising, bull's eye rots (*Pz. malicorticis*), and bitter rot (*C. gloeosporioides*) reported here are novel information. The method improved the eradication effect and increased residual activity of heating and CA storage, because it caused significantly lower decay than heated apples stored in air or than nonheated apple stored in CA.

Apple defense systems against fungi, which comprise either complex constitutive or nonconstitutive interactions, can be improved by heating (Ben-Yehoshua et al., 1998). Heat treatment was previously found to inhibit respiration rate and ethylene pro-

duction (Lurie et al., 1995). Our study showed that heat treatment caused an acceleration of CO<sub>2</sub> production in the first week of storage, which recovered during the subsequent storage period, but had no such effect on ethylene production. From the fifth week until the end of the storage, both parameters remained lower in the heat-treated fruits than in the nonheated. Thus, heat treatment delayed ripening and softening and thereby improved apple resistance to fungal infection during storage (Janisiewicz et al., 2003; Saffner et al., 2003). Heat treatment may also enhance host resistance to fungal attack by physiological or structural changes in the fruit surface. Fungal spores and latent infections occur either in the fruit surface or in the few cell layers under the apple peel. Cracks, wounds, and other breaks in the fruit surface are therefore the most

important vents for hyphal penetration (Porat et al., 2000; Schirra et al., 2000). Nonheated fruit have an interconnected network of numerous deep surface cracks in their epicuticular wax, whereas the epicuticular wax of heat-treated fruit has no such network (Lurie et al., 1996; Roy et al., 1994). In this study, we observed significant changes in epicuticular wax resulting from heat treatment with melted wax filling microcracks; covering germinated spores, conidia, and hyphae; and preventing inoculation. Differing cultivar responses to heating may be the result of variations in skin wax amount and structure. Apples with heavier and hardened epicuticular wax layers, containing few or no cracks, showed better resistance to pathogen attack, whereas apples with a clumpy wax layer showed better response to heat treatment. Nail-like wax

crystals needed higher temperatures to melt and protect against decay. Heat treatment melted wax platelets into cracks and did not recrystallize them, as reported previously (Roy et al., 1994).

From the provided data, it could be concluded that heat treatment at 40 °C and exposure period for 24 h in combination with CA storage is a promising strategy for increasing resistance to bruising and fungal decay in both cv. Aroma and cv. Ingrid Marie. This method provided acceptable protection against bull's eye rot (*Pz. malicortici*s) and bitter rot (*C. gloeosporioides*) and shortened the exposure period.

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