Effect of controlled atmosphere on postharvest quality of 'Douradão' peaches

Efeito da atmosfera controlada na qualidade pós-colheita de pêssegos 'Douradão'

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Abstract

This study was carried out with one of the most important cultivar grown in the State of Sao Paulo, Brazil, which has gained the preference of consumers, due to its sweet taste, intense skin color and large size; however, these fruits are susceptible to chilling injury when cold stored for long periods. The use of controlled atmosphere (CA) with elevated CO_2 and reduced O_2 concentrations prevent the onset of the chilling symptom. Thus, the effect of three different conditions of controlled atmosphere (CA1, CA2, CA3 and Control) was evaluated in order to extend the storage life of 'Douradão' peaches. After 14, 21 and 28 days, samples were withdrawn from CA and kept in fresh air at $25 \pm 1\ °C$ and $90 \pm 5\%$ RH to complete ripening. On the day of removal and after 4 days, were the peaches quality characteristics were evaluated. The results showed that the use of CA during cold storage reduced weight loss and prevented postharvest decay. CA2 and CA3 treatments were effective in keeping good quality of 'Douradão' peaches during 28 days of cold storage, the ripe fruits showed reduced incidence of woolliness, adequate juiciness and flesh firmness. CA1 and Control treatments did not present marketable conditions after 14 days of cold storage.

Keywords: Prunus persica; chilling; woolliness; storage life; pectolytic enzymes.

Resumo

Este estudo foi realizado em uma das mais importantes cultivares produzidas no Estado de São Paulo, Brasil, que tem a preferência dos consumidores devido ao sabor doce, intensa coloração e tamanho grande; entretanto, estes frutos são susceptíveis a danos pelo frio quando estocados sob refrigeração por longos períodos. O uso de atmosfera controlada (elevadas concentrações de CO2 e baixas de O2) previne os sintomas deste distúrbio. Foi avaliado o efeito de três condições de atmosfera controlada (AC1, AC2, AC3 e Controle) para estender a vida de estocagem dos pêssegos 'Douradão'. Após 14, 21 e 28 dias, amostras foram retiradas da AC, mantidas em ar a $25 \pm 1\ °C$ e $90 \pm 5\%$ UR para completar o amadurecimento. No dia da remoção e após 4 dias, foram avaliadas as características de qualidade dos pêssegos. Os resultados mostraram que a AC reduziu a perda de massa e preveniu a podridão pós-colheita dos frutos. Os tratamentos AC2 e AC3 foram efetivos na manutenção da boa qualidade dos pêssegos 'Douradão' durante 28 dias de armazenamento, os frutos maduros apresentaram reduzida incidência de lanosidade, adequada suculência e firmeza da polpa. Os tratamentos AC1 e Controle não apresentaram condições de comercialização após 14 dias de frigoconservação.

Palavras-chave: Prunus persica; injúria; lanosidade; vida de estocagem; enzimas pécticas.

1 Introduction

Peaches (*Prunus persica* L) 'Douradão' are one of the most important cultivar grown in the State of Sao Paulo, Brazil, whose production has increased in the past years. These fruits have gained the preference of consumers, who have mentioned it as having better qualitative characteristics such as sweet taste, intense skin color and large size, as well as of traders and their clients, mainly due to the higher prices tendency. However, nonripe fruits are subject to chilling injury when exposed to cold temperature (lower than 5 °C) for more than 14 days; woolliness is the main symptom, the fruit becomes dry and mealy or woolly in texture. This disorder is not externally visible; fruits are often marketed at this stage, which can lead to decreased consumers' acceptance.

Formation of woolliness has been associated to impaired solubilization of pectic substances (BEN-ARIE; LAVEE, 1971). The authors have indicated that pectin methylesterase enzyme demethylates pectins during low temperature storage, what results in an insoluble low methoxyl pectin of high molecular weigh that can form gel substance with free water in cellular fluid. Abnormal polygalacturonase activity has been associated with uncommon pectin metabolism, including the accumulation of insoluble pectins in cell wall. Thus, anomalous pectolytic enzymes action, induced by chilling, could account for impaired depolymerization of pectins and concomitant development of

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woolliness (BEN-ARIE et al., 1989; FERNÁNDEZ-TRUJILLO; CANO; ARTÉS, 1998; ZHOU et al., 2000).

Reports has related that the use of controlled atmosphere (CA) with elevated CO_2 and reduced O_2 concentrations delays or prevents the onset of this chilling symptom and the storage life of peach can be extended (LILL; DONAGHUE; KING, 1989; KADER, 2003; LURIE; CRISOSTO, 2005). Cell wall changes after CA storage appeared to be similar to the changes during ripening without storage, where woolliness did not develop (LURIE et al., 1994).

Kader (2003) reported that since 1997 there have been a few modest increases in the commercial use of CA during transport of several commodities in the world. CA storage has increased because it permits the harvest of some fruit when they are more fully-mature (higher yield), facilitates the use of lower temperature (<5 °C) without chilling injury, which allows for non-chemical insect control in some commodities, for markets that have restrictions against pests endemic to exporting countries and for markets that prefer organic produce. The author recommended continued technological development in the future to provide CA during transport at a reasonable cost are essential to expanded application on fresh fruits.

According to Kader (2002), the effects of CA such as delay senescence, decreased respiration rate and ethylene production, retard growth of decay-causing pathogens, depending on fruit variety or cultivar, physiology state, atmosphere composition, storage temperature and time. Currently, there is no information about the use of CA to prevent the chilling injury of 'Douradão' peaches and to extend their storage life. Thus, the objective of this study was to evaluate the effect of CA on postharvest quality of cold stored 'Douradão' peaches.

2 Materials and methods

2.1 Material and experimental design

Peaches (*Prunus persica* L) 'Douradão' were harvested from a commercial orchard in Jarinu, Sao Paulo, located in the southeast of Brazil under a sub-tropical climate; they were pre-selected and transported at ambient temperature for about 80 km to the Postharvest Laboratory (ITAL, Campinas/SP, Brazil). Fruits were selected according to size and skin background color (green-yellow), at commercial maturity, and immediately pre-cooled to 5 °C. Before pre-cooling, 72 peaches were randomly sampled and placed in 6 polypropylene (PP) trays (United Plastic Corporation S.A., Santiago, Chile), containing 12 peaches each, which were held in fresh air at 25 ± 1 °C and 90 ± 5% RH for 4 days for ripening. On the same day of harvest (3 trays) and on the 4th day of ripening (3 trays, each tray constituted a replicate), the peaches were evaluated for weight loss, decay incidence, flesh firmness, woolliness incidence, soluble solids content, titratable acidity, juice content and pectolytic enzymes activities. Three fruits from each tray were frozen in liquid nitrogen and held at –20 °C until enzyme assays.

The experiment was carried out in an entirely randomized design, where fruits were randomly distributed into four lots;

each lot containing 72 peaches was placed in hermetically closed 15 L chambers. The CO_2 and O_2 concentrations inside the chambers were modified and established through a continuous flow system - the gas mixtures were supplied from high pressure cylinders. The gas concentrations were monitored every two days during cold storage using a gas analyzer (PBI Dansensor, Combi-check 9800-1, Ringsted, Denmark). The following treatments were tested:

- (T_1) Control: fresh air (0.03 kPa CO_2 and 20.9 kPa O_2).
- (T_2) CA1: 3.0 kPa CO_2 and 1.5 kPa O_2 (balance N_2).
- (T_3) CA2: 5.0 kPa CO_2 and 1.5 kPa O_2 (balance N_2).
- (T_4) CA3: 10.0 kPa CO_2 and 1.5 kPa O_2 (balance N_2).

The fruits from all treatments were stored for 28 days at 1 ± 1 °C and 90 ± 5% RH. The room temperature was monitored by an electronic thermostat and the fruit temperature by a mercury thermometer (0.1 °C accuracy) inserted in fruit flesh. After 14, 21 and 28 days, the chambers were taken from cold storage, the fruits from each treatment were distributed in 6 PP trays and subsequently were held in fresh air at 25 ± 1 °C and 90 ± 5% RH for 4 days for ripening. Quality parameters were determined on the same day of removal and on the 4th day of ripening, as mentioned to the fruits at harvest. Three replicates per treatment were obtained to each assayed period (each tray containing 12 peaches constituted a replicate).

2.2 Quality evaluation

Weight loss was expressed as percentage of initial mass weight. The mass of each replicate was determined using a digital scale with an accuracy of 0.01 g (Gehaka, model PG2000, Sao Paulo, Brazil).

Decay incidence (DI) was determined visually (adapted from BASSETTO, 2006) in the fruits from three trays and rated as 0 = absent; 1 = very slight (1% ≤ surface ≤ 10%); 2 = slight (10% < surface ≤ 25%); 3 = moderate (25% < surface ≤ 50%); 4 = severe (50% < surface ≤ 100%). Healthy fruit were those showing no signs of decay. The DI was calculated as follows: DI = (number of healthy fruit × 0) + (number of fruits with very slight decay × 1) + (number of fruits with slight decay × 2) + (number of fruits with moderate decay × 3) + (number of fruits with severe decay × 4), divided by 4 × N (N = total number of fruits).

On each day of evaluation, ten fruits were randomly removed from three trays for determination of ripeness characteristics. The fruits were cut into two halves and woolliness incidence (WI) was determined visually on both sides (adapted from FERNÁNDEZ-TRUJILLO; CANO; ARTÉS, 1998) and rated as very slight (1% ≤ area ≤ 10%); slight (10% < area ≤ 25%); moderate (25% < area ≤ 50%); severe (50% < area ≤ 100%). Healthy fruit were those showing no signs of internal breakdown. The WI was calculated as mentioned for decay incidence.

Flesh firmness was measured on two opposite sides of each fruit using a TAXT-2 Texture Analyzer (Stable Micro Systems LTD, Surrey, England) at room temperature, equipped

with a 8 mm tip diameter plunger, operating at the following conditions: 5.0 mm/second pre-test speed, 1.0 mm/second test speed, 5.0 mm/second post-test speed, 9 mm depth and 25% strain (AMERICAN..., 2000).

Segments from each fruit were homogenized using a commercial blender (Philips Walita, R16720, Brazil) and soluble solids content (SSC) was measured with a hand refractometer (Atago, model N-1α, Tokyo, Japan), expressed as °Brix. Titratable acidity (TA) was determined by titrating 10 g of the homogenized pulp with 0.01 N NaOH to endpoint of pH 8.1 (Micronal Titrator, model B274, Sao Paulo, Brazil), the results were expressed as g of malic acid in 100 g^{-1} sample (ASSOCIATION..., 1995). The ratio between soluble solids content and titratable acidity was determined. The amount of extractable juice was determined using a refrigerate centrifuge (Sorvall Inc., RMC14, Norwalk, USA), where 1.5 g of homogenized pulp from each fruit was transferred into an Eppendorf tube and centrifuged at $6,000 \times g$ for 15 minutes, the weight of the supernatants was expressed as a percentage of the weight of the fresh fruit (adapted from VON MOLLENDORFF; DE VILLIERS, 1988a).

2.3 Pectolytic enzymes activities

For enzyme extraction, 150 g of frozen fruit were ground for 2 minutes in a homogenizer with 100 mL of cold aqueous solution containing polyethylene glycol 12% (PEG 4000, Merck, Darmstadt, Germany) and 0.2% sodium bisulfite. The homogenates were centrifuged (Beckman Centrifuge, Model J2-21, Beckman Instruments Inc., Palo Alto, CA., USA) at $10,000 \times g$ for 15 minutes at 5 °C, the pellet was collected and separated into two parts for extraction of enzymes.

For polygalacturonase (PG, EC 3.2.1.15) extraction, the pellet was incubated on a shaker (Marconi, Dubnoff mod. 145, Brazil) at 4 °C for 2 hours in cold 50 mM Na acetate buffer (Merck, Brazil), pH 5.0 and 0.5 M NaCl; after centrifugation as above, the supernatant was used as crude extract. The endo-PG activity was measured in a Cannon-Fenske viscosimeter (Model N.100, USA) by mixing 3 mL of enzyme extract with 4.5 mL 2% polygalacturonic acid (Sigma Chemical Company, G-2125, USA) in 50 mM Na acetate pH 4.4. Initial viscosity was measured and after a further 18-hour incubation at 30 °C (ZHOU et al., 2000). One activity unit was defined as the change in viscosity per g of fresh sample per hour. For determination of the exo-PG activity, 1 mL of extract was mixed to an equal volume of 2% polygalacturonic acid in 50 mM Na acetate buffer pH 4.4 and incubated at 30 °C for 18 hours. Reducing sugars released were determined through the Somogyi method (NELSON, 1944). Galacturonic acid was used as standard, and controls of boiled extract were run. One activity unit was defined as 1 μg galacturonic acid released per g of fresh sample per hour. For pectin methylesterase (PME, EC 3.1.1.11) extraction, the pellet was suspended in cold 7.5% NaCl and 0.75% EDTA pH 6.5 solution, that was incubated on a shaker at 4 °C for 2 hours; the supernatant was collected after centrifugation. For determination of PME activity, 1 mL of extract was mixed to 20 mL of 1% citric pectin (Sigma Chemical Company, P-9135, USA) solution containing 0.2 N NaCl, pH adjusted to 7.4

with 0.1 N NaOH and it was titrated (Mettler-Toledo titrator, DL50 Grafix, Columbus, OH., USA) to maintain pH 7.4 for 30 minutes, while incubated in a thermostatic bath (MLW, model U-2, Germany) at 30 °C (ZHOU et al., 2000). One unit activity was calculated as 1 μM NaOH consumed per g of fresh sample per hour.

2.4 Statistical analysis

An ANOVA for each quality attribute was performed using the F test and the treatment means were compared by the Tukey multiple range test at $p \leq 0.05$, using the SAS statistical package (Statistical Analysis System, 2003). The values at harvest and after each storage period were compared to find significant differences among treatments. The percentage data for the variable woolliness and decay incidence were normalized according to the Equation 1.

$$f(x) = arcsen (x + 0.5)^{1/2} \tag{1}$$

3 Results and discussion

3.1 Quality evaluation

Weight loss and decay incidence

No significant difference in weight loss was detected during the cold storage period, fruits from all treatments showed 0.3 to 0.6% weight loss (Table 1). After an additional 4-day ripening, weight loss of Control treatment increased to approximately 6.5 to 7.5%, which was increasing according to advancing cold storage period and differed significantly from CA treatments. Fruits from CA storage reached about 4.5 to 5.5% weight loss and no differences between these fruits and those ripened immediately after harvest were detected. Previous reports also related beneficial effect on reducing weight loss in post-storage ripening of peaches maintained at CA during cold storage (RETAMALES et al., 1992; MITCHELL; CRISOSTO, 1995; NAVA; BRACKMANN, 2002).

CA had positive influence on decay prevention of 'Douradão' peaches. Fruits from Control treatment showed an increase of this index according to advancing storage time, reaching 25.0% rotten fruit (identified as *Monilinia fructicola* G. Wint) on ripening after 28 days of cold storage, that performances high economic loss (Table 1). Fruits from CA cold storage presented about 5.0% decay incidence, after same period. These results are supported by reports from other authors on CA effects on reducing decay (RETAMALES et al., 1992; NAVA; BRACKMANN, 2002; GIRARDI et al., 2005).

Flesh firmness

CA had great influence on the maintenance of flesh firmness; CA2 and CA3 treatments (higher CO_2 concentrations) maintained flesh firmness after 28 days of cold storage. However, whatever the treatment with post-storage ripening, there was a drastic decrease in flesh firmness. The lower values were found in Control and CA1 treatments, which differed significantly from CA2 and CA3 treatments, after 28 days of cold storage (Table 1).

Table 1. Weight loss, decay incidence and flesh firmness of 'Douradão' peaches after harvest and under CA after cold storage (CS) at 1 ± 1 °C during 14, 21 and 28 days, plus 4 days ripening in fresh air at 25 ± 1 °C.

	Weight loss (%)					
After		+ 4 d ripening		+ 4 d ripening		+ 4 d ripening
Harvest	-	5.46[x] ± 0.23[yAB]	-	5.46 ± 0.23 [B]	-	5.46 ± 0.23 [B]
Treatments	After 14 d CS	+ 4 d ripening	After 21 d CS	+ 4 d ripening	After 28 d CS	+ 4 d ripening
Control	0.43 ± 0.10 [a]	6.45 ± 0.55 [A]	0.58 ± 0.10 [a]	6.71 ± 0.70 [A]	0.65 ± 0.10 [a]	7.32 ± 0.47 [A]
CA1	0.32 ± 0.10 [a]	5.17 ± 0.27 [B]	0.47 ± 0.10 [a]	6.15 ± 0.67 [AB]	0.54 ± 0.10 [a]	6.34 ± 0.44 [B]
CA2	0.51 ± 0.10 [a]	4.97 ± 0.53 [B]	0.50 ± 0.10 [a]	5.51 ± 0.59 [AB]	0.62 ± 0.10 [a]	5.63 ± 0.40 [B]
CA3	0.43 ± 0.10 [a]	5.26 ± 0.83 [B]	0.38 ± 0.10 [a]	5.90± 0.51 [AB]	0.50 ± 0.10 [a]	6.02 ± 0.46 [B]
	Decay incidence (%)					
After		+ 4 d ripening		+ 4 d ripening		+ 4 d ripening
Harvest	0,0 ± 0.0 [a]	5.21 ± 0.36 [A]	0,0 ± 0.0 [b]	5.21 ± 0.36 [B]	0,0 ± 0.0 [b]	5.21 ± 0.36 [B]
Treatments	After 14 d CS	+ 4 d ripening	After 21 d CS	+ 4 d ripening	After 28 d CS	+ 4 d ripening
Control	0,0 ± 0.0 [a]	6.25 ± 0.24 [A]	3.13 ± 0.14 [a]	18.75 ± 3.98 [A]	6.25 ± 0.24 [a]	25.00 ± 3.61 [A]
CA1	0,0 ± 0.0 [a]	4.17 ± 0.28 [A]	0,0 ± 0.0 [b]	5.21 ± 0.36 [B]	1.04 ± 0.09 [b]	6..38 ± 1.08 [B]
CA2	0,0 ± 0.0 [a]	0,0 ± 0.0 [B]	0,0 ± 0.0 [b]	2.08 ± 0.5 [B]	0,0 ± 0.0 [b]	5.21 ± 0.36 [B]
CA3	0,0 ± 0.0 [a]	0,0 ± 0.0 [B]	0,0 ± 0.0 [b]	2.08 ± 0.5 [B]	0,0 ± 0.0 [b]	5.21 ± 0.36 [B]
	Flesh firmness (N)					
After		+ 4 d ripening		+ 4 d ripening		+ 4 d ripening
Harvest	54.27 ± 2.94 [ab]	5.39 ± 0.77 [A]	54.27 ± 2.94 [a]	5.39 ± 0.77 [A]	54.27 ± 2.94 [a]	5.39 ± 0.77 [A]
Treatments	After 14 d CS	+ 4 d ripening	After 21 d CS	+ 4 d ripening	After 28 d CS	+ 4 d ripening
Control	46.50 ± 5.85 [b]	4.84 ± 0.92 [A]	42.38 ± 8.71 [b]	3.95 ± 0.46 [B]	34.25 ± 9.46 [b]	3.67 ± 0.45 [C]
CA1	55.75 ± 4.72 [a]	5.09 ± 0.97 [A]	48.93 ± 7.80 [ab]	4.81±0.57 [AB]	46.26 ± 7.90 [a]	4.28 ± 0.41 [BC]
CA2	56.57 ± 5.93 [a]	5.46 ± 0.85 [A]	53.45 ± 5.85 [a]	5.41±0.76 [AB]	50.96 ± 5.72 [a]	5.14 ± 0.47 [A]
CA3	53.43 ± 4.79 [ab]	5.41 ± 0.62 [A]	55.14 ± 6.54 [a]	5.11±0.84 [AB]	49.22 ± 8.94 [a]	4.62 ± 0.55 [AB]

[x]Average and [y]Standard deviation; Control = air; CA1 = 3 kPa CO_2 + 1.5 kPa O_2; CA2 = 5 kPa CO_2 + 1.5 kPa O_2; CA3 = 10 kPa CO_2 + 1.5 kPa O_2; For each parameter in the column, same letters do no differ significantly from each other, according to Tukey test ($p \leq 0.05$); Ratio (SSC/TA).

Control treatment did not present marketable conditions with respect to flesh firmness, allied to weight loss. Other reports (RETAMALES et al., 1992; ZHOU et al., 2000; NAVA; BRACKMANN, 2002; ROMBALDI et al., 2002; GIRARDI et al., 2005; GIEHL, 2006) related lower degradation of flesh firmness in the ripe fruits, after cold storage under CA when compared to control treatment.

In general, ratio values presented no significant difference during cold storage, except for CA3 treatment that differed from Control after 21 days of cold storage. With the exposure at room temperature, an increase in ratio values was verified in all treatments; no significant differences between them were found, except the CA3 treatment, that continued differing from Control after 21 days of cold storage plus four days of ripening (Table 2).

Fruits evaluated after harvest presented higher ratio values than cold storage fruits, probably cold temperature delayed fruit ripening; however, no significant differences were found between treatments and fruits after harvest, except for the CA1 treatment after fourteen days of cold storage plus four days of ripening and the CA3 treatment after twenty-one days of cold storage plus four days of ripening.

In this study, no significant difference was found between the CA treatments for titratable acidity, whatever the storage period (data not shown); there was no clear influence of CA on the titratable acidity of 'Douradão' peaches. Similar tendencies were found by other authors (RETAMALES et al., 1992; NAVA; BRACKMANN, 2002; GIRARDI et al., 2005) in their studies on acidity of peaches under CA cold storage; in post-storage ripening no difference was detected between treatments.

Murray et al. (1998) and Lurie; Crisosto (2005) observed that the increase of soluble solid content depends on the maturity grade of the peach and storage conditions; the authors mentioned that CA cold storage can delay the initiation of changes in chemical and biochemical attributes in these fruits. Other studies on CA cold storage with different peach and nectarine cultivars (ZHOU et al., 2000; GIRARDI et al., 2005) mentioned that the fruits stored in CA showed lower values of SSC when compared to control fruit.

Juice content and woolliness incidence

The juice content of the fruits decreased in all treatments according to advancing cold storage period. After 28 days, CA2 and CA3 treatments presented higher juice content and differed significantly from Control and CA1 treatments. With post-storage ripening, an increase in juice content was detected in all treatments. The lower juice content of the fruits found in Control and CA1 treatments, differed significantly from the CA2 and CA3 treatments, that no differed of those fruits evaluated after harvest, except after 28days (Table 2). Other reports (ZHOU et al., 2000b; GIRARDI et al., 2005; GIEHL, 2006) related higher juice content on ripe fruit from CA when compared to control fruit.

Table 2. Ratio (SSC/TA), juice content and woolliness incidence of 'Douradão' peaches after harvest and under CA after cold storage (CS) at 1 ± 1 °C during 14, 21 and 28 days, plus 4 days ripening in air at 25 ± 1 °C.

	Ratio (SSC/TA)					
After		+ 4 d ripening		+ 4 d ripening		+ 4 d ripening
Harvest	$30.62^x \pm 3.78^{y\,a}$	47.21 ± 2.98^A	30.62 ± 3.78^{ab}	47.21 ± 2.98^A	30.62 ± 3.78^a	47.21 ± 2.98^A
Treatments	After 14 d CS	+ 4 d ripening	After 21 d CS	+ 4 d ripening	After 28 d CS	+ 4 d ripening
Control	30.80 ± 3.81^a	38.93 ± 3.18^{AB}	42.25 ± 3.17^a	45.92 ± 3.06^A	30.26 ± 3.35^a	46.44 ± 2.58^A
CA1	27.75 ± 2.75^a	32.90 ± 2.61^B	34.35 ± 3.08^{ab}	41.78 ± 3.99^{AB}	32.80 ± 2.67^a	43.53 ± 3.57^A
CA2	29.29 ± 3.68^a	39.09 ± 3.04^{AB}	29.50 ± 3.74^{ab}	40.76 ± 2.06^{AB}	33.33 ± 3.19^a	55.78 ± 3.80^A
CA3	27.20 ± 2.91^a	36.53 ± 3.90^{AB}	28.03 ± 3.92^b	34.09 ± 3.20^B	31.09 ± 3.94^a	46.52 ± 2.81^A
	Juice content (%)					
After	At harvest	+ 4 d ripening	At harvest	+ 4 d ripening	At harvest	+ 4 d ripening
Harvest	65.36 ± 3.34^a	73.98 ± 2.79^A	65.36 ± 3.34^a	73.98 ± 2.79^A	65.36 ± 3.34^a	73.98 ± 2.79^A
Treatments	After 14 d CS	+ 4 d ripening	After 21 d CS	+ 4 d ripening	After 28 d CS	+ 4 d ripening
Control	63.61 ± 3.42^a	66.50 ± 4.41^B	59.40 ± 4.66^a	61.79 ± 3.07^C	53.52 ± 2.93^b	56.66 ± 2.32^D
CA1	65.72 ± 3.02^a	69.59 ± 4.45^{AB}	62.26 ± 3.74^{ab}	63.48 ± 2.69^C	57.59 ± 3.30^b	58.67 ± 2.52^D
CA2	66.12 ± 1.51^a	72.28 ± 4.51^A	66.23 ± 3.48^a	71.29 ± 2.60^{AB}	65.26 ± 2.29^a	69.67 ± 2.27^B
CA3	65.59 ± 2.60^a	70.37 ± 2.62^{AB}	66.39 ± 2.66^a	68.65 ± 4.63^B	64.95 ± 3.84^a	66.22 ± 2.60^C
	Woolliness incidence (%)					
After		+ 4 d ripening		+ 4 d ripening		+ 4 d ripening
Harvest	0.0 ± 0.0^c	0.0 ± 0.0^C	0.0 ± 0.0^c	0.0 ± 0.0^C	0.0 ± 0.0^c	0.0 ± 0.0^C
Treatments	After 14 d CS	+ 4 d ripening	After 21 d CS	+ 4 d ripening	After 28 d CS	+ 4 d ripening
Control	37.50 ± 0.53^a	37.50 ± 0.69^A	45.83 ± 3.61^a	66.67 ± 7.55^A	68.75 ± 6.83^a	93.75 ± 6.25^A
CA1	16.67 ± 0.15^b	33.33 ± 0.86^B	33.33 ± 6.22^b	39.58 ± 7.22^B	54.17 ± 5.55^b	68.75 ± 6.25^B
CA2	0.0 ± 0.0^c	0.0 ± 0.0^C	0.0 ± 0.0^c	0.0 ± 0.0^C	6.25 ± 0.0^c	6.25 ± 0.0^C
CA3	4.17 ± 0.52^c	0.0 ± 0.0^C	6.25 ± 6.22^c	8.33 ± 3.61^C	6.25 ± 0.52^c	12.50 ± 0.0^C

[x]Average and [y]Standard deviation; Control = air; CA1 = 3 kPa CO_2 + 1.5 kPa O_2; CA2 = 5 kPa CO_2 + 1.5 kPa O_2; CA3 = 10 kPa CO_2 + 1.5 kPa O_2; For each parameter, in the column, same letters do no differ significantly from each other, according to Tukey test ($p \leq 0.05$).

The use of CA reduced the occurrence of woolliness in 'Douradão' peach during cold storage. Control treatment showed more than 37% woolly fruit after 14 days at cold storage, increasing this index according to advancing storage time, reaching 68% woolly fruit after 28 days at cold storage; in this same period CA2 and CA3 treatments were little affected and reached only 6% wooly fruit. The CA1 treatment reached 54% wooly fruit, after 28 days at cold storage (Table 2). Several studies about woolliness in peaches and nectarines reported that this condition became apparent by the time the fruits were removed from cold storage and kept at 20-25 °C (CRISOSTO; MITCHELL; JU, 1999; ZHOU; BEN-ARIE; LURIE, 2000; ROMBALDI et al., 2001). With post-storage ripening, Control treatment showed 93% woolly fruit after four weeks of cold storage, while CA2 and CA3 treatments reached lower woolliness incidence, 6 and 12%, respectively; CA1 treatment showed 68% woolly fruit in the same period. Previous studies reported on CA effects on reducing woolliness incidence in peaches (BEN-ARIE; SONEGO, 1980; LURIE; CRISOSTO, 2005; GIRARDI et al., 2005) and in nectarines (ZHOU et al., 2000), support our results.

3.2 Pectolytic enzymes activities

In CA cold storage, there was no significant difference in fruit concerning the exo-PG activity between treatments, whatever the storage period. With post-storage ripening, an increase in exo-PG activity was observed in all treatments, although these values were always lower than the ones of the fruit evaluated after harvest. After 21 and 28 days of cold storage, the exo-PG activity of CA stored fruits was higher and differed significantly from Control treatment (Table 3). Several studies on the biochemical basis have identified factors which may be important in the development of woolliness symptoms, although many discrepancies exist among these studies. One of these factors is exo-PG activity, that was mentioned as having reduced activity in woolly fruit by some authors (ZHOU et al., 2000; GIRARDI et al., 2005) or peaches that developed woolliness during ripening showed an increase on exo-PG activity (VON MOLLENDORFF; DE VILLIERS, 1988b) or other studies reported that there was no relation with woolliness symptoms (ARTÉS; CANO; FERNÁNDEZ-TRUJILLO, 1996; BRUMMELL et al., 2004).

In the same way, in CA cold storage fruits, no significant difference was found in the endo-PG activity between the treatments, whatever the storage period. With post-storage ripening, an increase in endo-PG activity was observed in all treatments, although these values were lower in Control and CA1 treatments, which differed significantly from CA2 and CA3 treatments, after 21 and 28 days of cold storage (Table 3). Previous studies (GIRARDI et al., 2005; ZHOU et al., 2000) reported higher endo-PG activities in 'Chiripá' peach fruit and 'Flavortop' nectarine fruit cold stored at CA (5.0 kPa CO_2 + 1.5 kPa O_2 and 10.0 kPa CO_2 + 3.0 kPa O_2, respectively), after 5 days ripening at 20 °C. The authors related that the temperature

Table 3. Exo-PG, endo-PG and PME activity of 'Douradão' peaches after harvest and under CA after cold storage (CS) at 1 ± 1 °C during 14, 21 and 28 days, plus four days ripening in air at 25 ± 1 °C.

	Exo-PG (µg galacturonic acid g^{-1} sample h^{-1})					
After		+ 4 d ripening		+ 4 d ripening		+ 4 d ripening
Harvest	42.90 ± 2.76^{a}	88.39 ± 4.48^{A}	42.90 ± 2.76^{a}	88.39 ± 4.48^{A}	42.90 ± 2.76^{a}	88.39 ± 4.48^{A}
Treatments	After 14 d CS	+ 4 d ripening	After 21 d CS	+ 4 d ripening	After 28 d CS	+ 4 d ripening
Control	40.04 ± 3.69^{a}	58.46 ± 2.46^{B}	37.09 ± 3.69^{a}	53.43 ± 3.74^{C}	35.61 ± 3.97^{a}	55.50 ± 1.04^{C}
CA1	39.06 ± 1.90^{a}	56.98 ± 4.44^{B}	38.96 ± 4.58^{a}	64.46 ± 1.88^{B}	39.75 ± 1.94^{a}	60.92 ± 1.68^{BC}
CA2	33.54 ± 3.29^{a}	66.04 ± 5.13^{B}	36.10 ± 3.73^{a}	69.68 ± 3.16^{B}	38.57 ± 1.80^{a}	67.51 ± 4.05^{B}
CA3	37.97 ± 2.52^{a}	62.79 ± 3.58^{B}	35.61 ± 3.35^{a}	68.80 ± 2.10^{B}	37.88 ± 3.01^{a}	63.08 ± 4.75^{BC}
	Endo-PG (change in viscosity (s) g^{-1} sample h^{-1})					
After		+ 4 d ripening		+ 4 d ripening		+ 4 d ripening
Harvest	0.14 ± 0.016^{a}	0.30 ± 0.004^{A}	0.14 ± 0.016^{a}	0.30 ± 0.004^{A}	0.14 ± 0.016^{a}	0.30 ± 0.004^{AB}
Treatments	After 14 d CS	+ 4 d ripening	After 21 d CS	+ 4 d ripening	After 28 d CS	+ 4 d ripening
Control	0.12 ± 0.096^{a}	0.16 ± 0.012^{C}	0.12 ± 0.006^{a}	0.15 ± 0.002^{C}	0.11 ± 0.024^{a}	0.14 ± 0.016^{C}
CA1	0.12 ± 0.017^{a}	0.23 ± 0.009^{B}	0.12 ± 0.012^{a}	0.20 ± 0.021^{B}	0.12 ± 0.007^{a}	0.14 ± 0.020^{C}
CA2	0.12 ± 0.007^{a}	0.23 ± 0.050^{B}	0.12 ± 0.002^{a}	0.30 ± 0.008^{A}	0.13 ± 0.015^{a}	0.32 ± 0.013^{A}
CA3	0.12 ± 0.029^{a}	0.22 ± 0.017^{BC}	0.12 ± 0.007^{a}	0.28 ± 0.010^{A}	0.13 ± 0.013^{a}	0.28 ± 0.007^{B}
	PME (µM NaOH g^{-1} sample h^{-1})					
After		+ 4 d ripening		+ 4 d ripening		+ 4 d ripening
Harvest	76.37 ± 3.06^{ab}	56.49 ± 2.69^{D}	76.37 ± 3.06^{c}	56.49 ± 2.69^{D}	76.37 ± 3.06^{c}	56.49 ± 2.69^{E}
Treatments	After 14 d CS	+ 4 d ripening	After 21 d CS	+ 4 d ripening	After 28 d CS	+ 4 d ripening
Control	81.69 ± 1.16^{a}	92.79 ± 3.28^{A}	100.78 ± 3.17^{a}	108.31 ± 1.43^{A}	108.56 ± 1.16^{a}	110.75 ± 4.38^{A}
CA1	72.30 ± 1.94^{b}	70.56 ± 2.23^{B}	89.96 ± 1.87^{b}	89.18 ± 3.44^{B}	90.45 ± 1.99^{b}	100.65 ± 1.88^{B}
CA2	70.54 ± 1.08^{bc}	63.36 ± 3.13^{C}	72.83 ± 2.64^{c}	67.95 ± 2.14^{C}	72.50 ± 2.12^{c}	71.38 ± 1.04^{D}
CA3	64.82 ± 4.04^{c}	60.15 ± 2.73^{CD}	74.26 ± 2.35^{c}	73.66 ± 4.11^{C}	77.66 ± 2.33^{c}	86.64 ± 2.24^{C}

[x]Average and [y]Standard deviation; Control = air; CA1 = 3 kPa CO_2 + 1.5 kPa O_2; CA2 = 5 kPa CO_2 + 1.5 kPa O_2; CA3 = 10 kPa CO_2 + 1.5 kPa O_2; For each parameter, in the column, same letters do no differ significantly from each other, according to Tukey test ($p \leq 0.05$).

and the ethylene levels reduction had more effect on the endo-PG and exo-PG activities than on the PME activity.

In CA cold storage, fruits showed significant difference in the PME activity between treatments, lower values were presented by CA2 and CA3 treatments for all storage periods. With post-storage ripening, a decrease in PME activity was observed in these treatments, while Control and CA1 treatments presented an increase in PME activity (Table 3). Numerous studies have associated woolly fruit with PME activity, although they have presented different conclusions. Woolliness has been related to the reduction of PME activity (BUESCHER; FURMANSKI, 1978) or to an increase (BEN-ARIE; SONEGO, 1980; BRUMMELL et al., 2004; GIRARDI et al., 2005) or to expressionless levels of this enzyme (ZHOU et al., 2000).

In this study, it was observed that the reduction on the O_2 partial pressure and the increase on CO_2 partial pressure (above 5.0 kPa) had a pronounced effect in the induction of endo-PG and exo-PG, and in the inhibition of PME activity of 'Douradão' peaches. Previous reports (BEN-ARIE; SONEGO, 1980; ZHOU et al., 2000; GIRARDI et al., 2005; LURIE; CRISOSTO, 2005) associated the incidence of woolliness in peaches to an imbalance between PG and PME activities. The authors mentioned that when the imbalance occurs, the pectin is deesterified (due to high PME activity) without subsequent degradation (due to low PG activity), resulting in large pectin molecules with reduced methylation, that can form gel with

free water in cellular fluid, leading to less juice content and to the development of woolliness.

Another observed fact; there was a significant increase in polygalacturonase activity at the 4th day ripening in the CA2 and CA3 treatments, which showed lower firmness degradation (Table 1). Although the softening of fruit is correlated to the increase of PG activity, studies on transgenic tomato with reduced levels (1% of common type) of polygalacturonase showed that fruit apparently softened normally (SMITH et al., 1988). In other experiment with transgenic tomato, the over-expression of polygalacturonase in the non-softening mutant material failed to induce softening (GROSS, 1991). Thus, despite the strong correlation between polygalacturonase activity and fruit softening, in our study, the higher PG activity did not correspond to softer fruit; therefore, polygalacturonase alone may not be the unique responsible cause to induce fruit softening.

4 Conclusions

CA had influence on reducing weight loss and preventing decay incidence, but no relevant effect on ratio (SSC/TA) values.

CA of 5.0 kPa CO_2 and 1.5 kPa O_2 and also 10.0 kPa CO_2 and 1.5 kPa O_2 at 1 °C were effective in keeping the good quality of 'Douradão' peaches during 28 days of storage. These CA also had positive effect on the induction of polygalacturonase activity and inhibition of pectin methylesterase activity, and the

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ripe fruits showed reduced incidence of woolliness, adequate juiciness and flesh firmness. CA of 3.0 kPa CO_2 and 1.5 kPa O_2 was ineffective in keeping the good quality of fruits, which showed lower flesh firmness and higher woolliness incidence during storage life.

Control fruit did not present marketable conditions after 14 days of cold storage. These fruit presented a drastic increase in softening, significant weight loss and decay incidence, higher woolliness index and lower juice content.

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