Proceedings VI World Avocado Congress (Actas VI Congreso Mundial del Aguacate) 2007.
Viña Del Mar, Chile. 12 – 16 Nov. 2007. ISBN No 978-956-17-0413-8.

EFFECT OF TEMPERATURE BREAK IN THE BEHAVIOR OF AVOCADOS (*Persea americana* Mill.) HASS cv. DURING REFRIGERATED STORAGE

P. Undurraga[1], J. A. Olaeta[1] and J. San Martín[1]

[1] Facultad de Agronomía. Pontificia Universidad Católica de Valparaíso. San Francisco s/n La Palma Quillota. Chile. Correo electrónico: pundurra@ucv.cl

To evaluate the effect caused by a temperature break on the quality and conservation of stored fruit, avocados of Hass cultivar were harvested with a stage of ripeness between 10 – 12% of oil, weighted, evaluated the resistance of their pulp to pressure, color, presence of pathogens, physiological disorders and enzymatic analysis, and then refrigerated at 7°C (45 °F) ± 1°C (34 °F) and 90 – 95% de relative humidity, for 30 and 40 days in Hass. At days 7 and 15 of refrigerated storage, the fruit was subjected to a temperature break for 48 and 72 hours at 25°C (77°F). At the end of each storage period, the following was evaluated: weight loss, pulp resistance to pressure, epidermis color, physiological disorders, pathological damages and enzymatic analysis; this was also evaluated at the beginning and at the end of the application of cold break, analysis of which was only carried out to those treatments with a cold break for 3 days. Subsequently, the fruit was left to soften at room temperature up to 1,81 kg of pressure, with the same variables being evaluated again. In Hass cultivar, cold breaks for 2 or 3 days cause weight loss, early softening, with absence of pathogens and physiological disorders. Cold breaks for 3 days in Hass cause the fruit to reach 40 days of refrigeration in bad conditions as well as the control.

Key words: Quality, Softening, Physiological disorders, Maturity.

EFECTO DEL QUIEBRE DE TEMPERATURA EN EL COMPORTAMIENTO DE PALTA (*Persea americana* Mill.) CV. HASS EN ALMACENAMIENTO REFRIGERADO

P. Undurraga[1], J. A. Olaeta[1] y J. San Martín[1]

[1] Facultad de Agronomía. Pontificia Universidad Católica de Valparaíso. San Francisco s/n La Palma Quillota. Chile. Correo electrónico: pundurra@ucv.cl

Para evaluar el efecto que produce un quiebre de temperatura sobre la calidad y conservación de la fruta en almacenamiento, paltas del cv. Hass fueron cosechadas con un estado de madurez entre 10 – 12% de aceite, pesadas, y luego refrigeradas a 7ºC ± 1ºC y 90 – 95% de humedad relativa, por 30 y 40 días. A los 7 y 15 días de almacenamiento refrigerado, la fruta fue sometida a un quiebre de temperatura a 25ºC por 48 y 72 horas. Al inicio del ensayo y al término de cada periodo de almacenamiento, se evaluó: pérdida de peso, resistencia de la pulpa a la presión, color de epidermis, desórdenes fisiológicos, daños patológicos y análisis enzimático. Este último se evaluó además, al inicio y término del quiebre de frío por 72 horas. Posteriormente, la fruta se dejó ablandar a temperatura ambiente hasta 1,81 k de resistencia de la pulpa a la

presión, volviéndose a evaluar las mismas variables descritas. Quiebres de frío por 48 ó 72 horas, producen pérdida de peso y ablandamiento prematuro, sin presencia de patógenos ni desórdenes fisiológicos. Quiebres de frío por 72 horas, hace que la fruta llegue en malas condiciones a los 40 días de refrigeración, al igual que el testigo.

Palabras claves: calidad, ablandamiento, desórdenes fisiológicos, madurez

1. Introduction

Avocados are mainly transported by sea to their destinations for at least 25 days to Europe and 32 to Japan (CSAV, 2006). Therefore, it is essentially important to keep optimum storage conditions during transport.

In case of a possible increase in exports to farther markets, knowing the effects of failures that may occur in the storage systems becomes fundamental regarding the postharvest life duration and the organoleptic quality that fruits may reach.

Within this context, according to Canessa (2006), cold breaks produced in Hass avocado after 15 days of refrigerated storage with temperatures up to 15°C, for a maximum of 2 days, do not reduce the postharvest life and final quality of avocados harvested with 10 – 15% oil, until 30 days of refrigerated storage at 7º C ± 1º C and with 90 – 95% relative humidity. However, cold breaks on day 15 of refrigerated storage at 25°C for 2 days tend to cause a faster softening once avocados have been removed from the storage, reducing its commercialization period.

In addition, cold breaks generated on day 15 of refrigerated storage at 25°C for a period of 1 or 2 days cause a greater weight loss with fruit harvested with 10 - 15% oil, with a storage period of 20, 25 and 30 days at 7º C ± 1º C and 90-95% relative humidity (Canessa, 2006).

In the present trial, the effect of temperature and cold break period were evaluated on the shelf life and final quality of Hass avocados, harvested with 10 - 12% oil, on days 30 and 40 of refrigerated storage.

2. Materials and methods

The Hass avocados were harvested with a maturity level of 10 - 12% of oil on October 3rd, 2006, at the Experimental Station La Palma of the Faculty of Agricultural Sciences, Pontificia Universidad Católica de Valparaiso, located in Quillota (Latitude 32° 49' S, Longitude 71° 16'W). The percentage of oil was determined by obtaining the dry weight. A total of 375 fruits were collected.

Subsequently, the fruits were taken to laboratory to evaluate the following: weight (electronic balance PRECISA 3100C), pulp resistance to pressure (penetrometer EFFEGI FT 011 of stem 8 mm in diameter), color (colorimeter MINOLTA CR-200 and values expressed in CIE Lab and modified by Mc Guire, 1992), pathological

damage (occurrence/absence), physiological disorders (occurrence/absence) and enzymatic analysis (pectinmethylesterase and polygalacturonase). Then, the fruits were refrigerated at 7 ± 1ºC and 90 – 95% of relative humidity, for 30 and 40 days.
On day 7 of refrigerated storage, 150 fruits were divided in 2 groups of 75 fruits each, which were subjected to a temperature break for 48 and 72 hours at 25°C, respectively. Then, on day 15 of refrigerated storage, other 150 fruits were divided in 2 groups of 75 fruits each, being subjected to a temperature break for 48 and 72 hours at 25°C, respectively. After the temperature breaks, the fruits are taken back to chamber with the initial conditions, taking as control 75 fruits without temperature break.

After each storage period, the same variables measured at the beginning of the trial were evaluated. The enzymatic analysis was carried out during the following phases: at the beginning of the trial, at the moment of conducting the temperature break for 72 hours, at the end of this and when finishing each storage period.

After each storage period, the fruit was left to soften at ambient temperature up to 1.81 k of pulp resistance to pressure, evaluating again the same variables above-mentioned. In addition, a sensory evaluation panel was formed to determine taste, texture and color, with the following arbitrary scale: 1= Very bad; 2= Bad; 3= Regular; 4= Good; 5= Very Good.

The activity of polygalacturonase was determined through the capacity of galacturonic acid released by the enzymatic action to reduce dinitrosalicylic acid (DNS). A polygalacturonic acid (substrate) solution was mixed in acetate buffer pH 4.0 with a certain volume of enzymatic extract. It was left to react for 10 minutes at 30°C. The reaction was stopped when 2 ml of DNS were added. It was cooled down and centrifuged at 720 g for 20 minutes. The absorbance was determined at 540 nm. The activity of the enzyme was expressed in: (µmol / min of galacturonic acid released) / µg ptna (Menéndez *et al*., 2006).

To determine the activity of the pectinmethylesterase enzyme, carboxy groups released in a citrus pectin solution (substrate) at 1% in NaCl 0.1M, to which enzymatic extract was added, were titrated. The pH of the reaction mixture was kept at pH 7.5 for 5 minutes by addition of NaOH 0.01 N, with continuous stirring at 30°C through a magnetic stirrer. The activity of the enzyme was expressed in: milliequivalent of hydrolyzed ester / (ml of extract x min of reaction) (Rouse and Atkins, 1995).

For the variables of weight loss, resistance to pulp pressure and color, a Completely Randomized Multifactorial Design of 2x3x3 (2 periods of cold break x 3 times of refrigerated storage before the break x 3 times of refrigerated storage). For the enzymatic analysis, a factorial design 2x7x2 was used (2 days of break x 7 days of storage x 2 times before the temperature break). The experimental unit for the variables weight loss, pulp resistance to pressure and color were 5 avocados with 5 replications; whereas for the enzymatic analyses 4 replications of 5 avocados each. The variables were analyzed through analysis of variance for

the Fisher's F-test. If differences were detected among the treatments, mean separation was made through Tukey Test ($p \leq 0.05$). The variables of taste, texture and color were analyzed by the non-parametric Kruskal-Wallis test ($p \leq 0.05$).

3. Results and discussion

For the variable of pulp resistance to pressure and weight loss, it was determined that only the interaction between the duration of cold break and storage period had a significant effect. The effect of days of cold storage prior to temperature break was not significant.

Table 1 shows the interaction effect on pulp resistance to pressure and on weight loss. Regarding the pulp resistance to pressure, no significant differences among the different treatments were observed during the same evaluation dates, except for the control evaluated on day 30 of storage. These results coincide with Opazo's (2000), who obtained values of 6.03 k in refrigerated storage at 7 ± 1ºC for 30 days; however, on day 40 of refrigerated storage, Opazo determined that pulp resistance to pressure was 1.34 k.

In addition, Canessa (2006) obtained values above 12.42 k on day 30 of storage, applying a temperature break for 2 days at 25°C. This does not coincide with the results obtained in this trial, which may be due to the fact that these avocados were in the same chamber with those subjected to 25°C for 3 days, but the latter have generated the ethylene production, which is one of the responsible in the softening of avocados, since the maximum production of ethylene is obtained between 20 and 25°C (Eaks, 1978).

Zauberman and Jobin-Decor (1995) state that the firmness of the fruit is reduced by 50% after 3 weeks of storage at 8°C, which coincides with the obtained result in this trial.

The weight loss increased during storage. This increase may be caused when exposing the avocados to 25°C, where the vapor pressure goes down in the ambient, elevating the vapor deficit gradient, which makes the fruit lose the free water faster. This was proved by Eaks (1978), who after keeping the fruit under a 5°C regime for 2 weeks, with a weight loss of 2.4 ± 0.2%, increased the temperature to 20°C for 5 days, and the moisture loss went up to 8.4 ± 0.4 %.

In this sense, Canessa (2006) obtained levels of weight loss around 5.3% on day 30 of refrigerated storage, by making temperature breaks for 2 days at 25°C.

Furthermore, Olaeta, Undurraga and Guajardo (2003) report on Isabel avocados with 12% oil a weight loss of 5.19% on day 40 of storage, which is similar to the results of this trial.

Table 1: Effect of the interaction between the temperature break (0, 2 and 3 days) and the period of refrigerated storage (0, 30 and 40 days) on the pulp resistance to pressure (k)

Interaction	**PRP (k)**	**Weight loss (%)**
0 days of break /0 days of storage	12.42 a	0.0a
0 days of break /30 days of storage	9.52 b	1.3a
0 days of break /40 days of storage	2.61 d	4.2 b
2 days of break /0 days of storage	12.42 a	0.0a
2 days of break /30 days of storage	6.15 c	1.2a
2 days of break /40 days of storage	2.36 d	5.8 c
3 days of break /0 days of storage	12.42 a	0.0a
3 days of break /30 days of storage	6.38 c	1.3a
3 days of break /40 days of storage	2.15 d	6.2 c

The same letters in the same column do not show any significant differences according to Tukey Test ($p \leq 0.05$).

Regarding the components of color (brightness (L), chroma (C) and hue angle (h°)), a significant triple interaction among duration of temperature break, days of refrigerated storage prior to temperature break and the time of refrigerated storage (Table 2) was observed.

Table 2: Effect of triple interaction among duration (days) of temperature break (0, 2 and 3 days), days of refrigerated storage prior to temperature break (7 and 15 days) and the time of refrigerated storage (0, 20 and 30 days), on brightness (L), chroma (C), and hue angle (h°)

Interaction	**L**	**C**	**hº**
0 days of break /0 previous days/0 days of R.S.	36.5 a	18.73 fg	127.03 f
0 days of break /0 previous days/30 days of R.S.	30.84 de	13.18 cdef	110.04 def
0 days of break /0 previous days/40 days of R.S.	26.36 f	6.96 a b	62.31 ab
2 days of break /7 previous days/0 days of R.S.	34.54 b c	16.52 defg	126.87 ef
2 days of break /7 previous days/30 days of R.S.	32.06 cd	11.94 bcd	112.94 ef
2 days of break /7 previous days/40 days of R.S.	26.77 f	6.16 a	68.85 abc
2 days of break /15 previous days/0 days of R.S.	38.24 a	20.05 g	126.62 ef
2 days of break /15 previous days/30 days of R.S.	27.45 ef	6.06 a	75.2 bc
2 days of break /15 previous days/40 days of R.S.	27.88 ef	7.81 abc	87.78 cd
3 days of break /7 previous days/0 days of R.S.	36.3 ab	17.93 efg	127.38 f
3 days of break /7 previous days/30 days of R.S.	30.98 cde	12.64 bcde	104.51 de
3 days of break /7 previous days/40 days of R.S.	25.71 f	6.07 a	58.07 ab
3 days of break /15 previous days/0 days of R.S.	35.95 ab	16.62 defg	126.14 ef
3 days of break /15 previous days/30 days of R.S.	26.54 f	6.06 a	48.31 a
3 days of break /15 previous days/40 days of R.S.	25.23 f	6.01 a	51.7 a

The same letters in the same column do not show any significant differences according to Tukey Test ($p \leq 0.05$).
R.S.: Refrigerated Storage

The brightness evaluated on the same dates shows similar values and does not exhibit significant differences in relation with the control, except for the treatment with 3 days of temperature break, 15 days prior to temperature break and evaluated on day 30 of storage. This suggests that the temperature breaks of 48 and 72 hours do not affect the brightness of Hass avocados.

Canessa (2006) obtained with temperature breaks for 2 days at 25°C on day 30 of refrigerated storage, an average L value of 32.26, similar to those obtained in this trial with 7 and 15 days of storage prior to break, 32.06 and 27.45, respectively.

Regarding chroma, the results obtained show that as fruits ripen, the color intensity decreases. A greater effect can be observed 15 days prior to break and with 30 days of storage, since the intensity decreases more and gets closer to the values obtained for the control and for the treatments on day 40.

With temperature breaks for 2 days at 25°C and previous 15 days of storage, Canessa (2006) obtained an average value of 13.95, which is quite higher than 6.06 obtained in this trial. However, these values are similar to those obtained in this trial with 7 days prior to break.

As for hue angle, the values obtained at the beginning of storage were 126 – 127 and after storage 48 - 51, that is, the h° value goes from the quadrant between 90° and 180° (yellow and bluish green, respectively) to 90° and 0° (yellow and purple red, respectively). It was observed that the treatments with 15 days of refrigerated storage prior to break show values of hue angle lower than on day 30. This could be provoked if the temperature breaks are applied in a later stage of storage; this causes the stimulation to chlorophyllases, making the fruit changing from green to purple.

Nevertheless, Canessa (2006) obtained values of h° of 126.23 by evaluating Hass avocados with temperature breaks for 2 days at 25°C on days 30 of refrigerated storage and 15 of storage prior to break. This does not coincide with the results obtained in this trial. This suggests that when applying temperature breaks in later maturity stages, more acute response in the change of fruit color is triggered.

For the activity of the polygalacturonase (PG) enzyme, it was only significant for the storage period, without affecting duration of temperature break, days of storage prior to break and interaction among the factors (Table 3).

Table 3: Effect of the evaluation period (0, 7, 10, 15, 18, 30 and 40 days) on the activity of polygalacturonase (PG) enzyme ((µmol / min of galacturonic acid) / µg ptna)

Evaluation period (days)	Activity PG
0	1.33 a
7	1.18 a
10	0.82 b
15	0.83 b
18	0.44 c
30	0.48 c
40	0.51 c

The same letters in the same column do not show any significant differences according to Tukey Test (p≤0.05).

The activity of PG decreased during storage, registering values of low activity since the evaluations were conducted with fruits just coming out of the chamber. Awad and Young (1979), and Zauberman and Schiffmann-Nadel (1972) indicate that PG does not show activity after the harvest of avocado and gradually increases as the fruit gets close to the climacteric peak; PG keeps increasing in the post-climacteric stage, playing an essential role in the softening of avocado.

The activity of this enzyme reflects that the softening process started early and decreased as the time went by. This may explain the values of pulp resistance to pressure obtained at the end of the refrigerated storage which were 2.3 k on day 40.

Unlike the obtained by PG enzyme, only interaction between the duration of temperature break and storage days was detected regarding the activity of pectinmethylesterase (PME) enzyme. The parameter of storage days prior to the application of the temperature break was not significant (Table 4).

Table 4: Effect of the interaction between the duration of temperature break (0 and 3 days) and the evaluation dates (0, 7, 10, 15, 18, 30 and 40 days) on the activity of the pectinmethylesterase (PME) enzyme (milliequivalent of hydrolyzed ester / (ml of extract x min of reaction))

Interaction	PME
0 days of break /0 days of storage	4.76 a b
0 days of break /7 days of storage	4.09 a
0 days of break /10 days of storage	5.61 a b
0 days of break /15 days of storage	4.93 a b
0 days of break /18 days of storage	16.42 c
0 days of break /30 days of storage	4.93 a b
0 days of break /40 days of storage	5.72 a b
3 days of break /0 days of storage	5.17 a b
3 days of break /7 days of storage	4.60 a b
3 days of break /10 days of storage	5.01 a b

3 days of break /15 days of storage	4.68 a b
3 days of break /18 days of storage	7.54 b
3 days of break /30 days of storage	4.59 a b
3 days of break /40 days of storage	4.95 a b

The same letters in the same column do not show any significant differences according to Tukey Test (p≤0.05).

The activity of the enzyme did not show significant variations within the whole storage period. The above mentioned does not follow the behavior patterns of this enzyme, since it shows a peak in harvest and decreases its rate as the time in storage passes, remaining in 70% of its initial activity during the pre-climacteric period; it falls abruptly to 20% of initial activity one day before the climacteric peak of respiration (Awad and Young, 1980).

Zaubermann and Jobin-Decor (1995) indicate that by storing Hass avocados at 8°C for a period of 4 weeks, the PME activity keeps constant, even once the temperature rises to 22°C to ripen. The above mentioned coincides with the obtained, since no differences are observed among the different interactions, except for the evaluations on day 18 that show higher activity.

The analysis of sensory evaluation (Table 5) showed that for the variables of pulp color, texture, for both day 30 and day 40 of refrigerated storage, in general the judges gave qualifications ranging from Regular to Good to the temperature break treatments conducted on days 7 and 15 of storage, not showing an effect on quality.

Regarding taste, the judges qualified as “Bad” most of the taste measures made on day 40 of storage, except for the treatments for 2 days of temperature break on days 7 and 15 days of storage. The taste on day 30 was qualified in general as “Good” to “Very Good.”

Table 5: Effect of the duration of temperature break (0, 2 and 3 days) and the days (0, 7 and 15) of refrigerated storage prior to temperature break on the organoleptic quality of Hass avocado evaluated on days 30 and 40 of refrigerated storage

Duration temperature break (days)	**Storage prior to break (days)**	**Pulp color**		**Texture**		**Taste**	
		30 days	**40 day**	**30 day**	**40 day**	**30 day**	**40 day**
0	0	3.8 a b	2.8 a	3.8 a b	2.4 a	3.4 a	1.8 a
2	7	3.2 b	3.4 a	3.2 c	3.2 a	3.8 a	3.8 c
2	15	4.4 a	3.4 a	4.4 a	3.0 a	5.0 b	2.8 b c
3	7	3.6 a b	3.4 a	3.4 b c	2.8 a	3.8 a	2.4 a b
3	15	3.6 b	3.2 a	3 c	3.0 a	3.2 a	2.2 a b

The same letters in the same column do not show any significant differences according to Fiedman Test (p≤0.05).
1= Very Bad; 2 = Bad; 3 = Regular; 4 = Good; 5 = Very Good

4. Conclusions

Cold breaks of 25°C on days 7 or 15 of refrigerated storage at 7°C ± 1°C with 90 - 95% relative humidity generate greater softening in the fruit on day 40 of refrigerated storage.

The hue angle and chroma show an effective reduction in evaluation on day 30 of refrigerated storage when temperature breaks are produced at 25°C on day 15 of refrigerated storage at 7°C ± 1°C with 90 – 95% relative humidity, regardless the duration of temperature breaks.

The weight loss, evaluated on day 40 of refrigerated storage at 7°C ± 1°C with 90 – 95% relative humidity, increases with cold breaks at 25°C, for 2 or 3 days, if these are generated on days 7 or 15 of cold storage.

Temperature breaks of 25°C for 2 days, produced on day 15 of refrigeration at 7°C ± 1°C with 90 – 95% relative humidity, in a sensory evaluation on day 30 of refrigerated storage, cause a very good taste of the fruit, situation that decays on day 40.

The Pectinmethylesterase enzyme in Hass avocado, stored at 7°C ± 1°C, when subjected to temperature break for 3 days at 25°C, does not show any response and keeps its activity constant; whereas polygalacturonase shows a decrease in its activity until 40 days of storage.

5. References

Awad, M. and Young, R. 1979. Postharvest Variation in Cellulase. Polygalaturonase. and Pectinmethylesterase in Avocado (*Persea americana* Mill. cv Fuerte) Fruits in Relation to Respiration and Ethylene Production. Plant Physiol 64. 306-308

Awad, M. and Young, R. 1980. Avocado Pectinmethylesterase Activity in Relation to temperature. Ethylene. and Ripening. J Amer. Soc. Hort. Sci. 105(5): 638-641

Canessa, P. 2006. Efecto del quiebre de temperatura en almacenaje refrigerado sobre la conservación y calidad organoléptica de paltas (*Persea americana* Mill) cv. Hass. en tres tiempos de almacenaje y en dos estados de madurez. p. 59. Taller de Licenciatura. Pontificia Universidad Católica de Valparaíso. Facultad de Agronomía. Quillota Chile.

CSAV. 2006. Itinerarios. Disponible en: http://csav.cl/pages/sp_schedules.htm. Leído el 27 de Marzo de 2006.

Eaks, I. 1978. Ripening. Respiration. and Ethylene Production of "Hass" Avocado fruits at 20° to 40° C. J. Amer. Soc. Hort. Sci. 103(5): 576-578.

Lizana, L.; Salas, M. y Berguer, H. 1992. The Influence of Harvest Maturity. Type of Parking and Temperatures on Avocado Quality. Proc. Of second avocado congress pp.435- 442.

Luza, J.; Berger, H. y Lizana, L. 1979. Almacenaje en frío de paltas (*Persea americana* Mill.) cvs. Negra de la Cruz. Ampolleta Grande y Fuerte. Simiente 49 (3-4): 42-47.

Menéndez, O, Evangelista, S., Arenas, M., Bermúdez, K., Martínez A. y Jiménez, A. 2006. Cambios en las actividades de alfa-amilasa. pectinmetilesterasa y poligalacturonasa durante la maduracion de maracuyá amarillo Pasiflora edulis var. Flavicarpa degener. Interciencia 31 (10): 728-733.

Olaeta, J.; Undurraga, P. y Guajardo, S. 2003. Caracterización y evaluación en almacenaje refrigerado del cv. de palto Isabel (*Persea americana* Mill). p. 659-664. In Proccedings V World Avocado Congreso. Granada. 19-24 de Octubre de 2003. Sociedad Internacional del Aguacate. Málaga. España.

Opazo, S. 2000. Caracterización histológica y bioquímica de desórdenes fisiológicos en paltas *(Persea americana* Mill.) cv. Hass en almacenaje refrigerado. en dos estados de madurez. Taller de licenciatura. Quillota. PUCV. 68p.

Rouse, A. and Atkins, C. 1955. Pectin esterease and pectin in Comercial Citrus jueces as determined by methods used at the citrus experiment station. Fla. Agric. Exp. Stn. Bull.. 570: 1-19.

Zauberman, G. and Jobin-Décor, M. 1995. Avocado (*Persea americana* Mill.) quality changes in response to low-temperature storage. Postharvest Biology and Technology 5: 235-243.

Zauberman, G. and Shiffmann-Nadel, M. 1972. Pectin Methylesterase and Polygalacturonase in Avocado Fruit in Various Stage of Development. Plant Physiol 49: 864-865.