



## Tolerance of codling moth, and apple quality associated with low pressure/low temperature treatments



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### ABSTRACT

A combination of low pressure (LP) and low temperature (LT) may serve as a phytosanitary disinfestation treatment for fresh fruit. In this study, different life stages of codling moth (eggs, 2nd to 3rd instar larvae, 5th instar larvae and pupae) were treated in hypobaric chambers maintained at 10 °C and 1.33 kPa with nearly saturated humidity (>98%). Weight loss, color, firmness, titratable acidity (TA), and soluble solids content (SSC) were selected as quality parameters to evaluate the quality changes of 'Red Delicious' apples before and after the LPLT treatment. Results showed that the 5th instar larvae were the most tolerant life stage for codling moth under LPLT treatment conditions. Insect mortality increased with increasing LPLT treatment time to >98% after 12 days of exposure to 10 °C temperature and 1.33 kPa pressure. Although stored in less than optimum conditions for apples, the measured quality variables of 'Red Delicious' were maintained relatively well after 15 days of LPLT treatment. The results suggest that LPLT technology has potential as an alternative, non-chemical disinfestation treatment for apples.

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### 1. Introduction

Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), a key pest of fresh fruit such as apples and pears (Witzgall et al., 2008), is an important phytosanitary and quarantine pest for many countries (Wang et al., 2004). Quarantine treatments using the chemical fumigant methyl bromide (MeBr) have been commonly required for importing countries to prevent the spread of this pest. Due to the ozone depletion potential of MeBr, its use is being phased out under the Montreal Protocol (UNEP, 2009). Most applications of MeBr have been banned in developed countries since the end of 2005 and will be banned worldwide by the end of 2015 (UNEP, 2009). Although quarantine and pre-shipment (QPS) treatments such as those targeting codling moth in fresh fruit, are currently exempt from restrictions under the Montreal Protocol, there is increasing pressure to extend the ban to these applications.

Consequently, it is necessary to explore alternative non-chemical disinfestation treatment methods.

Many disinfestation treatment methods have been considered as alternatives to MeBr, such as hot air or hot water treatments, radio frequency (RF) treatment, ionizing irradiation treatment, cold storage, controlled atmosphere storage and other fumigants (Heather and Hallman, 2008). Low-pressure technology has also been proposed as an alternative disinfestation treatment for agricultural products since it is organic, residue free and environmentally sustainable (Bare, 1948; Calderon et al., 1966; Calderon and Navarro, 1968; Mbata and Phillips, 2001; Navarro et al., 2001; Davenport et al., 2006; Johnson and Zettler, 2009). Most of the early studies on low-pressure technology for insect disinfestation focused on its use in durable commodities at relatively high temperatures (ambient or above) (Calderon et al., 1966; Mbata and Phillips, 2001; Navarro et al., 2001; Johnson and Zettler, 2009). In contrast, much of the research on low-pressure treatments for fresh fruit has been to maintain product quality while extending storage life. Those studies were conducted at low temperatures and high humidities (Burg, 2004). At low temperatures, longer exposures are needed to obtain adequate insect control with low pressures (Mbata and Phillips, 2001; Mbata et al., 2004, 2005). However, because low-pressure/low-temperature (LPLT) technology provides extended storage times for fresh fruit, it has the potential

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to be used as an alternative disinfestation treatment (Davenport et al., 2006).

The insecticidal mechanism of low-pressure treatments is thought to be primarily due to the effects of reduced oxygen levels (Navarro and Calderon, 1979). Soderstrom et al. (1990) reported the relative responses of different codling moth life stages to low oxygen conditions (0.5% O<sub>2</sub> and 10% CO<sub>2</sub> at 25 °C with 60% or 95% relative humidity), noting that diapausing larvae were the most tolerant. Johnson and Zettler (2009) investigated the response of three lepidopteron postharvest pests in tree nuts (codling moth, navel orangeworm and Indianmeal moth) under a low-pressure (6.67 kPa) environment at 25 and 30 °C temperatures. Information on the relative tolerance of different codling moth life stages under LPLT treatment conditions (low pressures, low temperatures and high humidity) is lacking. It is important to determine the most tolerant codling moth life stage under LPLT treatment conditions, and this stage must be used in developing and validating LPLT disinfestation treatment protocols.

In addition to determining the efficacy of the treatment in controlling the target pest, it is also necessary to determine the treatment effect on the product quality. When evaluating the stability and performance of the LPLT system under different pressures, Jiao et al. (2012) showed that the LPLT system had the ability to control the pressure within 1% of the set point and maintained relative humidity at a nearly saturated level (>98%). Oxygen concentrations could be controlled at low levels (<0.6%) when the pressure was less than 3.33 kPa. The leakage rates of the hypobaric chamber and of the entire LPLT system were 0.01 and 0.48 kPa/h, respectively, and were considered acceptable. The demonstrated performance of the LPLT system (Jiao et al., 2012) provides a solid basis for the current insect tolerance and fruit quality study.

The main objective of the present study was to investigate the feasibility of using the LPLT technique to control insects and maintain the quality of fresh fruit. The lab-scale LPLT system tested previously was used to study the tolerance of codling moth at different life stages under the LPLT treatment environment, and apple quality was evaluated before and after the LPLT treatments.

## 2. Materials and methods

### 2.1. LPLT systems

A lab-scale LPLT system (Atlas Technologies, Port Townsend, WA, USA) with two identical hypobaric aluminum chambers (0.61 L × 0.43 W × 0.58 H m<sup>3</sup>) was used in the current study. The system was equipped with a rotameter (Model FL-3841G, OMEGA Engineering Inc., Stamford, CT, USA) to adjust the air exchange rate, which was used to prevent buildup of metabolic gases given off by the fruit. A humidifier was used to make sure the inflowing rarefied air was humidified before entering the hypobaric chamber. Sensors inside the hypobaric chambers were used to record the temperature, humidity and pressure during treatment. The chamber system, housed in cold-storage room, was covered by flexible insulation sheets with a thickness of 0.013 m to reduce the temperature variations of the hypobaric chamber walls. Detailed information about the LPLT systems and instrumentation used in this study can be found in Jiao et al. (2012).

### 2.2. Insect mortality

Initial stock codling moths were obtained in 1984 from an apple orchard in Madera County, CA and reared at the San Joaquin Valley Agricultural Sciences Center (SJVASC). Test insects were reared at 27 °C, 60% RH and a photoperiod of 16:8 (L:D) h on agar-based lima bean diet in plastic 1 oz sample cups with snap on lids (SJVASC

Insectary, 2008). They were delivered to Washington State University (Pullman, WA, USA) by FedEx overnight shipment. Wang et al. (2002) found that overnight air-shipment of codling moth did not affect the viability of any of the tested life stages. Eggs were supplied on waxed paper strips fastened with double stick tape to the bottom of plastic Petri dishes. Post-embryonic stages were treated in diet cups when they were 1 week (2nd to 3rd instar), 2 weeks (5th instar), and 3 weeks (pupae) old.

All life stages of codling moths were exposed to the LPLT environment maintained at 10 ± 0.5 °C, 1.33 ± 0.03 kPa pressure and near saturated relative humidity. The LPLT system included two identical hypobaric chambers. For chamber #1, all test insects to be treated were placed in the chamber which was then sealed and brought to the target pressure of 1.33 kPa. The chamber was opened and samples of each targeted life stage were removed after 6, 8, 10, and 12 days of treatment. After each sample was removed the chamber was re-sealed and brought back to 1.33 kPa. The entire process of opening the chamber to remove a sample took less than 30 min. To evaluate the effect on insect mortality of opening the chambers to take out samples, test insects in chamber #2 were held at 1.33 kPa continuously for 12 days. Additional samples were held at normal atmospheric pressure (NAP) and 10 °C for 12 days to evaluate the effect of cold storage alone. Untreated insects held at room temperature (25 °C) were used as controls. In addition, egg samples were held at 28 °C, 60% RH and photoperiod of 14:10 (L:D) h at SJVASC as non-transit laboratory controls.

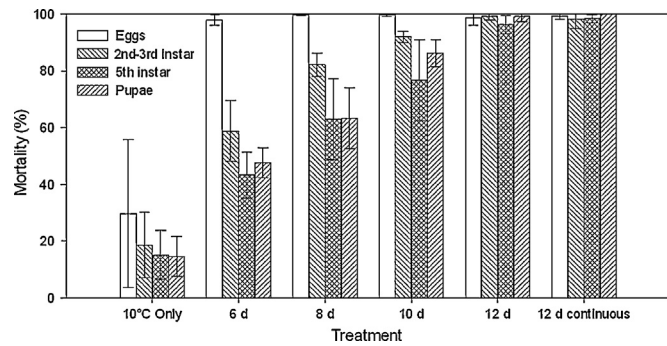
Immediately after treatment, a small amount (<1 g) of wheat bran based insect diet (SJVASC Insectary, 2006) was added to the egg dishes to provide food and humidity for hatching larvae. Dishes were held at 25 °C for at least 10 days after treatment and then frozen to kill any hatched larvae before returning them to SJVASC for observations. Egg mortality was calculated based on the percentage of unhatched eggs. Post-embryonic stages after treatment were held at 25 °C and a photoperiod of 14:10 (L:D) h until adult emergence. The number of treated insects was determined by counting the number of adults emerging from untreated room temperature controls. For each treatment, three dishes each containing about 50 eggs and 21 diet cups containing 34–44 larvae or pupae were used. The test was replicated three times, and mean values and standard deviations of insect mortality were calculated.

Insect mortality values from all treatments were analyzed using the least significant difference (LSD) *t*-test. Mortality data from the 6, 8, 10 and 12 d exposures for the post-embryonic stages were analyzed using the probit procedure in PoloPlus 2.0 (Robertson et al., 2003). Lethal exposure times for 50 and 95% mortality (LT<sub>50</sub> and LT<sub>95</sub>) were estimated for each post-embryonic stage. Estimated exposure times were compared among all life stages by using the lethal-dose ratio test in PoloPlus 2.0 (Robertson et al., 2003, 2007).

### 2.3. Quality evaluation

Red Chief 'Red Delicious' apples were obtained from the Washington State University Turkey orchard (Pullman, WA, USA). The apples were harvested at the climacteric state and then held in a cold storage room at around 2 °C and 90–95% relative humidity before the LPLT treatment. Samples of 50 apples (about 8.5 kg) were exposed to a LPLT environment of 1.33 kPa, 10 °C, and a nearly saturated humidity level (>98%) for 6, 9, 12 and 15 days. Additional apples were kept at room temperature (25 °C) or in the cold storage room for comparison with the LPLT treatment. Weight loss, color, firmness, titratable acidity (TA) and soluble solids content (SSC) were selected as the quality parameters to evaluate the quality of apples after the LPLT treatment. All treatments were replicated three times.

The weight loss percentages were calculated based on the initial weight of the apples. The skin color of the 'Red Delicious' apples



**Fig. 1.** The mortality of codling moth at four life stages (eggs, 2nd to 3rd instar larvae, 5th instar larvae and pupae) under different treatment methods and exposure times.

were measured by a chromameter (model CR-200, Minolta, Japan). Using an 8-mm measuring aperture, CIE  $L^*$ ,  $a^*$  and  $b^*$  values were recorded, and the chroma value was calculated from  $a^*$  and  $b^*$ . Apple firmness was determined as the maximum force required to push an 11 mm Magness–Taylor (MT) probe into the fruit flesh (after skin removal) to a depth of 8 mm (Topping, 1981; Fellman et al., 2003). The firmness results were expressed in Newton (N). For the color and firmness measurements, there were 9 data points for each measurement, mean and standard deviation values were calculated and used to evaluate the quality changes. The SSC was measured from the pressed juice of fruit by means of a hand refractometer (ATAGO Inc., Bellevue, WA, USA). The TA was determined by using 10 mL pressed juice, which was diluted with distilled water to 50 mL, 40 mL of diluted juice were titrated with 0.1 mol/L sodium hydroxide to a pH of 8.2. The results were expressed as percent malic acid. There were three replicates for each measurement for determining the TA and SSC.

Mean values and standard deviations were calculated from the replicates for each quality parameter under different treatments. The mean values were compared and separated at a significant level of  $P=0.05$  with the least significant difference (LSD)  $t$ -test using the Excel variance procedure (Microsoft Office Excel 2003).

### 3. Results and discussions

#### 3.1. Insect mortality

The mortality data for different codling moth life stages under different treatment conditions and exposure times are shown in Fig. 1. Because the number of treated post-embryonic test insects was estimated by the emergence of adults from untreated controls, no correction was made for control mortality for 2nd to 3rd instars, 5th instars and pupae. Egg mortality for each treatment was corrected for control mortality (Abbott, 1925). The estimated number of larvae and pupae for each treatment was 34–44 insects/replicate, and the number of treated eggs/replicate was 147–241.

No significant difference was observed between mortality of untreated laboratory control eggs (non-transit) and mortality of shipped untreated control eggs (transit) ( $P>0.05$ , data not shown), suggesting that the effect of overnight transportation

on egg mortality was minimal as observed for post-embryonic stages (Wang et al., 2002). There was also no significant difference ( $P>0.05$ ) in insect mortality between continuous and discontinuous treatments, which indicates that the influence of taking out the sample during LPLT treatment (<30 min) to insect mortality was negligible. After 12 days of storage at 10 °C and NAP, corrected mortality of codling moth eggs was around 30%, higher than that of the post-embryonic stages, which was less than 20% (Fig. 1). Egg mortality was also higher than that of the post-embryonic stages under 10 °C and 1.33 kPa. Average mortality of eggs was >98% while post-embryonic mortality was <60% after the shortest (6 d) exposure, indicating that eggs were the most susceptible to the LPLT environment (Fig. 1).

The results of the probit analysis for the post-embryonic stages are summarized in Table 1. Egg mortality was not included in the analysis because of high mortality levels at all exposures. Data from the 2nd to 3rd instar larvae were found to fit the probit model, while data for 5th instar larvae and pupae did not. Based on lethal dose ratio tests, there were no significant differences detected at the  $LT_{95}$  level, while  $LT_{50}$  for 2nd to 3rd instar larvae and pupae were significantly different ( $P<0.05$ ). However, lethal times for 5th instar larvae were consistently greater than 2nd to 3rd instar larvae or pupae. Consequently, 5th instar larvae were selected as the codling moth life stage most tolerant to LPLT treatment conditions, and will be used in subsequent studies.

Earlier work on the response of various stored product insects to low pressures has shown that eggs are commonly the most tolerant stage (Bare, 1948; Al-Azawi et al., 1983; Mbata and Phillips, 2001; Finkelman et al., 2003, 2004), but these studies usually were done at 18 °C or above. Davenport et al. (2006) found that Caribbean fruit fly eggs were the most resistant life stage under the LPLT treatments, where eggs could be controlled at probit 9 security within 9.4 days at 2.0 kPa pressure and 13 °C temperature. Johnson and Zettler (2009) showed that non-diapause codling moth larvae were less tolerant to 6.66 kPa at 25 and 30 °C than codling moth eggs at ambient humidities. Johnson (2010) reported that mortality of Indianmeal moth larvae to low pressure (6.66 kPa) was associated with moisture loss, and survival of larval stages increased with relative humidity. Our results indicate that codling moth eggs are less tolerant of exposure to 10 °C, and Yokoyama and Miller (1989) found that eggs were the life stage least tolerant to 0 °C exposures. The low temperatures and high humidities of our current study might, therefore, have reduced the relative tolerance of eggs while increasing that of the larval stages.

The mortality response of insects to a low oxygen environment is strongly affected by temperature; the exposures needed for effective control decrease as treatment temperature increases (Neven, 2003). Because hypoxia is the primary mortality mechanism for low-pressure treatments, the exposure needed for control also decreases with increasing temperature in all life stages (Mbata and Phillips, 2001; Mbata et al., 2005). Thus, the low temperature, coupled with the high relative humidity, applied in the current study increases the exposure time needed for insect control. However, those parameters have been shown to benefit fruit quality (Burg, 2004).

**Table 1**  
Lethal times (days) for different life stages of codling moth exposed to 1.33 kPa pressure, 10 °C, and near saturated humidity.

Stage	N	Slope ± SE	Chi square $\chi^2$	Heterogeneity	LT <sub>50</sub>	95% C.I.		LT <sub>95</sub>	95% C.I.	
						Lower	Upper		Lower	Upper
2nd to 3rd instar	555	0.319 ± 0.042	6.83	0.68	5.2 a	4.2	5.9	10.4 a	9.7	11.5
5th instar	585	0.290 ± 0.033	26.32	2.63	6.7 ab	5.2	7.6	12.4 a	11.0	15.4
Pupae	545	0.337 ± 0.038	11.64	1.16	6.5 b	5.7	7.1	11.4 a	10.6	12.8

Values in the same column with different letters are significantly different ( $P<0.05$ ; lethal dose ratio test).

**Table 2**  
Quality parameters of 'Red Delicious' apples under different treatments and exposure times over three replicates.

Quality parameters	Treatments	Treatment time (d)				
		0-Control	6	9	12	15
Color-L*	RT storage	70.9 ± 8.4 <sup>a, A</sup>	72.1 ± 9.3 <sup>a, A</sup>	72.2 ± 9.3 <sup>a, A</sup>	74.1 ± 10.0 <sup>a, A</sup>	73.9 ± 9.9 <sup>a, A</sup>
	0 °C storage	68.9 ± 9.2 <sup>a, A</sup>	67.9 ± 10.1 <sup>a, A</sup>	68.8 ± 9.4 <sup>a, A</sup>	68.3 ± 9.3 <sup>a, B</sup>	68.1 ± 9.9 <sup>a, B</sup>
	LPLT-ch1	68.8 ± 7.0 <sup>a, A</sup>	67.4 ± 7.8 <sup>a, A</sup>	67.5 ± 9.4 <sup>a, A</sup>	67.6 ± 8.4 <sup>a, B</sup>	67.2 ± 8.4 <sup>a, B</sup>
	LPLT-ch2	69.5 ± 8.2 <sup>a, A</sup>	-	70.4 ± 9.6 <sup>a, A</sup>	-	70.9 ± 10.2 <sup>a, A, B</sup>
Color-chroma	RT storage	37.6 ± 5.4 <sup>a, A</sup>	35.5 ± 6.5 <sup>a, A</sup>	36.1 ± 6.2 <sup>a, A</sup>	35.2 ± 6.2 <sup>a, A</sup>	35.6 ± 5.5 <sup>a, A</sup>
	0 °C storage	38.0 ± 5.4 <sup>a, A</sup>	39.7 ± 7.0 <sup>a, B</sup>	38.8 ± 6.2 <sup>a, A</sup>	39.3 ± 5.7 <sup>a, B</sup>	39.6 ± 6.5 <sup>a, B</sup>
	LPLT-ch1	39.0 ± 3.6 <sup>a, A</sup>	34.5 ± 4.2 <sup>b, A</sup>	34.2 ± 4.9 <sup>b, A, B</sup>	32.6 ± 5.6 <sup>b, A</sup>	32.0 ± 5.6 <sup>b, C</sup>
	LPLT-ch2	38.0 ± 4.6 <sup>a, A</sup>	-	32.4 ± 4.3 <sup>b, B</sup>	-	30.6 ± 5.0 <sup>b, C</sup>
Maximum force (N)	RT storage	72.7 ± 6.4 <sup>a, A</sup>	63.8 ± 7.9 <sup>b, A</sup>	62.8 ± 5.4 <sup>b, A</sup>	59.7 ± 4.6 <sup>b, A</sup>	59.4 ± 5.5 <sup>b, A</sup>
	0 °C storage	72.7 ± 6.4 <sup>a, A</sup>	71.2 ± 5.8 <sup>a, B</sup>	76.0 ± 7.2 <sup>a, B</sup>	71.8 ± 7.4 <sup>a, B</sup>	70.1 ± 8.5 <sup>a, B</sup>
	LPLT-ch1	72.7 ± 6.4 <sup>a, A</sup>	72.8 ± 7.0 <sup>a, B</sup>	72.7 ± 8.4 <sup>a, B</sup>	71.8 ± 8.1 <sup>a, B</sup>	70.0 ± 8.9 <sup>a, B</sup>
	LPLT-ch2	72.7 ± 6.4 <sup>a, A</sup>	-	74.5 ± 6.1 <sup>a, B</sup>	-	69.7 ± 9.8 <sup>a, B</sup>
TA (% malic acid)	RT storage	0.26 ± 0.05 <sup>a, A</sup>	0.22 ± 0.04 <sup>a, A</sup>	0.23 ± 0.05 <sup>a, A</sup>	0.23 ± 0.03 <sup>a, A</sup>	0.23 ± 0.05 <sup>a, A</sup>
	0 °C storage	0.26 ± 0.05 <sup>a, A</sup>	0.26 ± 0.04 <sup>a, A</sup>	0.27 ± 0.07 <sup>a, A</sup>	0.26 ± 0.06 <sup>a, A</sup>	0.27 ± 0.05 <sup>a, A</sup>
	LPLT-ch1	0.26 ± 0.05 <sup>a, A</sup>	0.25 ± 0.02 <sup>a, A</sup>	0.25 ± 0.04 <sup>a, A</sup>	0.23 ± 0.05 <sup>a, A</sup>	0.24 ± 0.03 <sup>a, A</sup>
	LPLT-ch2	0.26 ± 0.05 <sup>a, A</sup>	-	0.24 ± 0.03 <sup>a, A</sup>	-	0.27 ± 0.05 <sup>a, A</sup>
SSC (%)	RT storage	12.6 ± 0.5 <sup>a, A</sup>	13.4 ± 0.3 <sup>b, A</sup>	13.7 ± 0.5 <sup>b, A</sup>	13.4 ± 0.8 <sup>b, A</sup>	13.0 ± 0.8 <sup>a, A</sup>
	0 °C storage	12.6 ± 0.5 <sup>a, A</sup>	13.0 ± 0.7 <sup>b, B</sup>	12.8 ± 0.7 <sup>b, B</sup>	12.6 ± 0.7 <sup>a, B</sup>	13.1 ± 0.6 <sup>b, A</sup>
	LPLT-ch1	12.6 ± 0.5 <sup>a, A</sup>	13.0 ± 0.4 <sup>a, B</sup>	13.2 ± 0.3 <sup>a, A, B</sup>	12.9 ± 0.8 <sup>b, B</sup>	12.9 ± 0.3 <sup>a, A</sup>
	LPLT-ch2	12.6 ± 0.5 <sup>a, A</sup>	-	13.0 ± 0.7 <sup>b, B</sup>	-	12.9 ± 0.7 <sup>a, A</sup>

RT storage is room temperature storage.

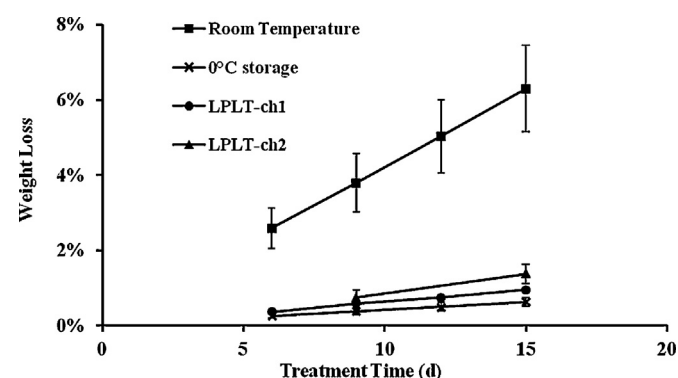
Values in the same row with different lower case letters were significantly different for different treatment time ( $P < 0.05$ ) for each quality parameter.

Values in the same column with different upper case letters were significantly different ( $P < 0.05$ ) among different treatments for each quality parameter.

### 3.2. Quality evaluation

Fig. 2 shows the weight loss of apples in different treatments and after different exposure times. Weight loss was the greatest when apples were kept at room temperature, with more than 6% weight loss after 15 days. Weight loss was much less in LPLT treatments compared with that at room temperature, and there were no significant differences between weight loss in LPLT treatment in chamber #1 and 0 °C cold storage after 15 days ( $P > 0.05$ ) (Fig. 2). For LPLT treatment in chamber #2, weight loss was significantly different ( $P < 0.05$ ) from 0 °C cold storage condition after 15 days storage. Generally weight loss after LPLT treatments was minimum, since the incoming air was humidified to achieve a high relative humidity inside the hypobaric chamber; this would help to keep weight loss to a minimum level if the relative humidity can be well controlled.

Table 2 lists all the quality parameters for 'Red Delicious' apples before and after different treatments with different exposure times. The experimental results indicate that there were no significant changes ( $P > 0.05$ ) for the  $L^*$  values after all treatments with different exposure times, suggesting that the lightness of the apple color



**Fig. 2.** The weight loss of "Red Delicious" apples exposed to different treatment conditions and treatment times.

was not very sensitive to the current treatments. However, Chroma values decreased with increasing exposure time under LPLT treatments (Table 2). The firmness of treated 'Red Delicious' apples was significantly reduced in the room temperature environment ( $P < 0.05$ ), probably caused by severe water loss. Water loss and cell wall breakdown were commonly considered as two major factors causing apple softening (Johnstone et al., 2002; Harsan et al., 2006). The firmness of apples was maintained well after 15 days at 0 °C cold storage or LPLT treatment conditions (Table 2). The TA and SSC values of treated 'Red Delicious' apples are also shown in Table 2. There were no significant changes ( $P > 0.05$ ) for TA under all treatments within 15 days. For SSC values, there were some variations under all treatments, but all values were within the range acceptable for 'Red Delicious' apples. No significant loss in the market quality of 'Red Delicious' and 'Golden Delicious' apples was found by Phillips et al. (2007) after a 5-day exposure to a low pressure (6.67 kPa) environment at 25 °C. Studies such as sensory evaluations are needed to further determine the effects of LPLT treatments on apple quality.

### 4. Conclusion

The results from our study suggest that the 5th instar was the most tolerant life stage and a lengthy 1.33 kPa, 10 °C LPLT treatment might be suitable for a phytosanitary treatment. Additional research, concentrating on the most tolerant life stage, is needed to provide probit 9 values. The quality of 'Red Delicious' apples was maintained after 15 days of the LPLT treatments. The current study showed that LPLT technology has the potential to be an alternative non-chemical disinfestation treatment method for apples, reducing the need for chemical fumigants while maintaining product quality and extending shelf-life.

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