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Immediate hydrocooling after phytosanitary hot-water treatment ensures mortality of third-instar larave of *Anastrepha ludens* without decreasing the fruit quality of mango cv. ´Ataulfo´ and ´Tommy Atkins´

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Abstract

Hydrocooling (HYC) after hot-water treatment (HWT) eliminates fruit fly larvae (Diptera: Tephritidae) and reduces the negative impact of HWT on mangoes. For HWT to be effective, the mangoes must be hydrocooled within 30 minutes after completing the HWT. If HYC is applied immediately after the HWT, an additional 10 minutes must be added to the hot water immersion to ensure larvae mortality. This experiment tested the effect of the HYC time (0, 10, 20, and 30 minutes) and HYC temperature (15.5, 21.1, 26.6, and 32.2 °C) on the survival of third-instar *Anastrepha ludens* and the quality of the mangoes. The mango varieties studied were cv. ´Ataulfo´ (weight < 425 g) and ´Tommy Atkins´ (weight < 500 g). The heating curves showed that the pulp reached 45°C by the end of the HWT in all cases. Treatments at temperatures between 46.4°C and 47.0°C, along with treatments at 15.5 to 21.1 °C, were effective for both mango varieties, with no survivors of third-instar *A. ludens*. We also noticed that the HWT produced a uniform color in mature fruits.

Key words: Mexican fruit fly, phytosanitary treatment, hot water immersion.

Introduction

In México, mangoes are grown in 224, 646 Ha with a production of 2, 299, 983 tons where the most common cvs ´Ataulfo´, ´Kent´, ´Manila´, and ´Tommy Atkins´ (SIAP 2024). The main producing zones are located in areas infested with *Anastrepha* fruit flies, and in order to export to fruit fly-free countries, a disinfestation treatment must be applied in a systemic approach (SENASICA 2025). The fruit fly disinfestation treatments are vapor heat, hot air, hot water, irradiation, quick freezing, fumigation and miscellaneous treatments (Johnson and Hofman 2009). The U.S. certification of mangos begins in the orchards, followed by a pre-screening of fruits in the packing sheds before the hot water treatment (HWT) (Alvarez 2006).

The HWT for *Anastrepha ludens* (Loew) (developed by Sharp *et al.*, 1989, and modified by Hernández *et al.*, 2007) requires mangoes of the cvs. ´Tommy Atkins´, ´Keitt´, ´Kent´, and ´Haden´ weighing 500 and 700 g to be treated for 75 and 90 min, respectively, in order to export them from Mexico to the United States. However, ´Ataulfo´ and ´Manila´ mangoes weighing 375 and 570 g require only 65 and 75 min, respectively. In all these cases, the water temperature must be ≥46.1 °C (115 °F), and the temperature of the pulp should reach 45 °C (113 °F (at the end of the treatment (USDA/SAGARPA, 2020; USDA/MRP/APHIS/PPQ, 2013). Protocol indicates that, to be sure the annihilation of larvae, HYC must be applied to fruits at least 30 minutes after HWT. However, to apply HYC immediately after the HWT, the dip time should be increased by 10 minutes (USDA/MRP/APHIS/PPQ, 2013). Hydrocooling (HYC) applied after a phytosanitary hot-water treatment (HWT) to control fruit fly larvae (Diptera: Tephritidae) reduces the negative effects caused by the HWT on mango fruits. As a result of the thermal conductivity of water, HYC rapidly reduces the effect of heat on mangoes, which helps preserve their quality. Hydrocooling also increases the shelf life of mangoes by restricting enzymatic and respiratory activity, reducing ethylene production, weight loss, and yellow index, retarding surface color changes and the decrease in fruit firmness, slowing the rate of water loss, and maintaining the soluble solids content (SSC) (Oosthuyse *et al.*, 1995, Jian *et al.*, 2012). However, immediately immersing the fruits in water at 21 °C may also increase the probability of survival of third-instar *A. ludens* (Shellie and Mangan, 2002a, 2002b).

Hallman and Sharp (1990) found that HYC at 21°C after HWT helped preserve mangoes quality; however, in subsequent treatments, one of 17, 589 *Anastrepha suspensa* (Loew) larvae survived in a batch of infested fruits, and thus the effectiveness of the treatment was called into question. In another study, Shellie and Mangan (2002a) observed that HWT at 46.1 °C for 65 to 75 min and HYC at 21°C applied to <400 g mangoes, result on less

of 100% *A. ludens* larval mortality, but HWT for 90 min with subsequent HYC applied to 687±17 g mango fruits resulted in 100% larval mortality. Hallman and Sharp (1990) and Shellie and Mangan (2002a, b) indicate that the heating curves of the different mango cultivars did not reach 45°C at the end of the HWT at 46.1°C. Thus, not reaching the target temperature may explain the larvae survival. Hernandez *et al.* (2017) showed that *A. ludens* third-instar larvae did not survive to HWT followed by HYC at 21 °C applied to ´Ataulfo´ or ´Tommy Atkins´ mangoes when the temperature at center of the fruits reached 45°C at the end of hot water dip. On the other hand, Hernández *et al.* (2017) demonstrated that third-instar *A. ludens* larvae in both "Ataulfo" and "Tommy Atkins" mangoes did not survive when HYC was applied immediately at 21 °C, especially after the fruit pulp's center reached 45 °C by the end of the HWT. The HYC increases the rate at which heat dissipates from the fruit, thereby reducing the exposure time of the larvae to lethal temperatures and decreasing their chances of survival. Shellie and Mangan (2012) stated that fruits are typically maintained at 45 °C for 10 minutes; however, this time requirement can be eliminated when HYC is applied right after the HWT.

If we assume that, under commercial conditions, the mango pulp remains at 45 °C for 10 minutes at the end of the hot water treatment (HWT) and no surviving larvae are observed, we can hypothesize that the factors influencing the effectiveness of the HWT, specifically the duration that the mangoes are held at 45 °C, are related to the temperature and duration of the hot water treatment (HYC). Also is important to determine whether fruit quality is affected. We addressed this by assessing the effectiveness of HWT combining four treatment times and four temperatures of HYC on the survival of third-instar larvae of *A. ludens* and the quality of mango fruits.

Materials and methods

Study site and biological material: The study was performed at the Escuela de Sistemas Alimentarios, Universidad Autónoma de Chiapas, located in Tapachula, Chiapas, Mexico. Export-quality mango batches, including cvs. ´Ataulfo´ (less than 425 g) and ´Tommy Atkins´(less than 500 g), were supplied by Empacadora Carrocera, located on the Suchiate-Jaritas highway in Tapachula, Chiapas. According to the regulations of the Mexican Mango Packers, the export-quality mangoes were early ripe fruits (commercial maturity), the selected fruits corresponded to those that presented pulp yellow without white portions, pericarp green and yellow, firmness of 18 kgf cm-2 and 8 °Brix (EMEX/ SAGARPA/ BANCOMEXT/ SE, 2005), which were selected according the epidermis color of 90-110 °Hue, determined using a Minolta CR-400 Chroma Meter colorimeter (8-mm aperture, D65 illuminant target reference).

Larvae of *A. ludens* were obtained from a wild colony founded with 6, 000 larvae from infested bitter orange fruits (*Citrus aurantium* L.) collected in Tapachula, Chiapas, Mexico during April 2023. The tests were performed during 2023 with the fourth and fifth generations of flies. The flies were kept under laboratory conditions, in the Escuela de Sistemas Alimentarios, Universidad Autónoma de Chiapas, in accordance with the methodology outlined by previous authors (Stevens, 1991; Orozco-Dávila *et al.*, 2017, 2020).

Experimental design and experimental unit: The efficacy of HWT+HYC was determined by analyzing the effect of HYC time and temperature on the survival of third-instar *A. ludens* larvae and fruit quality. A two-factor experimental design was established, where the first factor was cooling time (0, 10, 20, and 30 min) and the second factor was the temperature of the water (15.5, 21.1, 26.6, and 32.2 **ºC**) plus a Control (no-HWT), for a total of 20 treatments. Time 0 (zero) of HYC consisted of immersion of the fruits, without remaining submerged. Each experimental unit consisted of a fruit artificially inoculated with 40 larvae.

The fruits were artificially inoculated as described by Hallman (2000) and Shellie and Mangan (2002a,b), where two cavities were made in each fruit until the seed was reached using a 1.1-cm-diameter drill (Refacciones Agrícolas Industriales, Tapachula, Chiapas), resulting in two holes with a depth of 5 cm and a volume of 5.9 cm^3. Twenty larvae were placed in each cavity, which occupied a mean volume of ~2.77 cm^3, and each cavity was subsequently covered with a portion of mango pulp shaped into a cylinder using a no. 8 hollow punch (1.2 cm diameter × 0.5 cm height) and sealed with silicon glue (Truper glue sticks, Truper Herramientas, S.A. de C.V., Jilotepec, Mexico) using a Truper 12.7-mm hot melt glue gun (Hernández *et al.*, 2017). The infested fruits were kept at 26 **°C** and 65% RH for 24 h prior to the HYC tests. One replicate consisted of five infested fruits. Twelve replicates were carried out for each treatment, for which ten percent of the infested fruits were removed from each group, prepared for the corresponding treatment, and used to determine the effect of the experimental conditions on larval survival.

Treatments: Based on the experimental design described above, 20 treatments were created using various time and temperature combinations. The overall cooling time was 30 minutes, comprised of the cooling time on the platform (CTP) and the cooling time in the water (HYC). In these experiments, we considered as absolute control a group of fruits (20 fruits/ treatment) that were not treated with HWT or HYC, and were used to determine larval survival and fruit quality when the fruits are not subjected to phytosanitary treatments.

Hot-water and hydrocooling treatments: The fruits were kept in water at a temperature above 46.1 °C; it was necessary to pre-heat the water to 47 °C during the first five minutes. The water remained at 46.6 °C from minute 6 to minute 31, at 46.5°C from minute 32 to minute 50, and at 46.4 °C from minute 51 until the end of the treatment The cages with fruits were suspended above the tank for 5 to 10 minutes to drain the remaining water before the cooling treatment. For HYC, after HWT, the fruits were dipped at times and temperatures according to each treatment. The fruits treated were kept in laboratory conditions during 24 h. The total cooling time was 30 min, which corresponded to the sum of CTP and HYC.

Fruit temperature was recorded using SOFT-32 software connected to a telemetry module that had 8 channels and 8 TEL-MOD sensors connected to a PC interface and T001070 temperature sensors with ± 0.27 **°F** precision insertion points (CONTECH, Guadalajara, Jalisco, Mexico). All sensors were calibrated using a mercury thermometer with a temperature range from 75 to 125°F and precision of 0.1 **°C** as a reference standard, catalog number 3723RM-A-FC and NIST reference test 272630-06 (Brooklyn Thermometer Company Inc., Farmingdale, NY, USA). The equipment constructed on commercial specifications and requirements (Industrial Uruapan, Michoacán, Mexico). The equipment consisted of a 6.10-m long × 2.51-m wide × 1.85-m

deep Jacuzzi-type tank with a 21-m^3 volume and a capacity for 2 baskets with 80 boxes of fruit per basket. A Teledyne Laars series 8890578 gas-fired hot water boiler with 160 psi and a 0.2-m^3 heating surface was used to heat the water. The cooling tank (Coperlametic, 9RC1-1015-THC 505 B/M model, 3 HP, serial number 920 70290, 200/220 volts, 50 Hz, 42.8 R.L. A, 208 L.R.A.) (Construcciones y Servicios Industriales, Tepic, Nayarit, Mexico) had the same capacity as the hydrothermal tank. The equipment included a MEDA-TL automatic telemetry temperature controller with a RODEVA interface card, a MUX-A 8-channel multiplexer conditioning card, an SBC control card, a 20 × 24 LCD display, a KP-16 keyboard, a Smart SS solenoid card, an Emart FS light/switch card, and an SPS-1 commutation energy source.

Larval survival: One day after HWT+HYC, the infested fruits were dissected to extract and record the number of larvae. The recovered larvae were placed in Petri dishes with wet vermiculite and kept at 26 ºC and 70% RH to promote pupariation. Five days later, the pupae were separated from the vermiculite using a 14-mesh-per-inch sieve and placed in plexiglass cages of 30 × 30 × 30 cm for adult emergence. Only normal puparia (not showing any deformation) were counted and recorded (Thomas and Mangan 1997, Shellie and Mangan 2000a, b), and dead larvae and deformed puparia were removed to avoid microorganism contamination. Finally, survival rate was recorded based on the number of larvae that showed movement and managed to form a puparium. The number of surviving larvae was confirmed by the number of emerged adult flies from each treatment.

Fruit quality: The quality parameters of fruits were external color (° Hue), pulp color (° Hue), soluble solids (°Brix), acidity (%), firmness (kg-f), and weight loss (%) (Osuna-García *et al.*, 2002; Luna-Esquivel *et al.*, 2006; Siller-Cepeda *et al.*, 2009). Color was determined using a Minolta CR-400 Chroma Meter colorimeter (8-mm aperture, D65 illuminant target reference) based on the color saturation index or chromaticity, $Cr = (a^2 + b^2)^{½}$, and the hue angle, (°H) = $\tan^{-1} b \times a^{-1}$, where "a" represents the intensity of green or red and "b" the intensity of blue or yellow. Soluble solids were determined using an Atago PAL-α digital refractometer, 0 – 85% ºBrix, 0.1% resolution. Firmness was measured using a Tr® Forli – Italy penetrometer with an 11-mm piston. Acidity was determined following procedure 942.15B of AOAC (1998). Finally, weight loss was calculated by the equation described in Hernández *et al.* (2010),, where PP (%) = percentage of weight loss, P_i = initial weight, and P_f = final weight.

The quality parameters were assessed 14 days after the cooling treatment, when the fruits had reached maturity for consumption. A sample of six treated but no-infested fruits was used to measure the quality parameters.

Data analysis: The data of the number of individuals that survived to the HWT+HYC treatments are presented as a table. No statistical analysis was carried out in this case because, as expected, there were no larvae survivor in any treatment. Fruit temperature over time was plotted to show the differences among treatments.

A factorial analysis of variance (ANOVA) was used to evaluate the effect of the temperature and immersion time in hot water on the quality parameters of the fruits. Prior to the analysis, the data expressed as percentages were transformed by $\sin^{-1} [\sqrt{(x/100)}]$. A Tukey's test was applied to analyze the differences between treatment means using JMP Statistical Discovery Software (SAS Institute 2003). The level of significance was $P < 0.05$ in all cases. Surface graphs were made using the data of the quality parameters of the mangoes of both varieties, Ataulfo and Tommy Atkins using Statistica (data analysis software system) (2004). In addition, graphs fitted to a simple linear regression model were made using all data to describe the trend of each parameter during 21 days of shelf life.

Results

Efficacy of HWT+HYC: **Third-instar mortality**: A total of 40, 907 and 39, 736 larvae were treated with HWT+HYC in ´Ataulfo´ and ´Tommy Atkins´ mangoes, respectively. In control treatments, the survival was over 90%; while in all infested fruits treated, pupation was inhibited (Table 1), and alive larvae were not recorded. The heating curves showed that, in all treatments, pulp temperature of the mangoes cv. Ataulfo (Figures 1A-D) and Tommy Atkins (Fig. 2 A-D) reached a temperature of 45°C in 65 and 75 minutes, respectively. Furthermore, the duration for which the fruit remained at 45°C is variable. Notably, Tommy Atkins mangoes (Fig. 2A) do not maintain this temperature for a duration of 10 minutes when subjected to cooling in water at 15.5°C for 20 and 30 min. After completing the hot water treatment (HWT), the mangoes maintained a temperature of 45°C. In some cases, this temperature remained for an additional 5 to 10 minutes (Fig. 1A-D, Fig. 2A-D). This phenomenon is due to the time required to transfer the mango-filled baskets from one treatment tank to another. Furthermore, the baskets were deliberately suspended above the tank to allow residual water to drain. This crucial step ensures that the mangoes are properly prepared for the subsequent cooling process. Careful handling and timing during this phase are essential to preserving fruit quality.

Mango quality parameters: Ataulfo´ and ´Tommy Atkins´ mangoes cooled in water at 15.5 °C ripen more slowly according to external color, pulp color, total soluble solids, and firmness. Analysis of fruit quality parameters revealed distinct trends between both varieties Ataulfo (Fig. 3A-F) and Tommy Atkins (Fig. 4A-F). Specifically, the acidity and

Table 1. Number of larvae of the Mexican fruit fly, *Anastrepha ludens*, in ´Ataulfo´ *and ´Tommy Atkins´ mangoes* during the evaluation of different hydrocooling times and temperatures

Cooling time	Temperature				Survivors
	15.5 °C (°60F)	21.1 °C (°70F) (Control)	26.6 °C (°80F)	32.2 °C (°90F)	
	Third-instar larvae in treated ´Ataulfo´ mangoes				
0CTP - 30HYCT	2787	3286	2806	2384	No survivors
10CTP-20 HYCT	2709	2862	2853	2490	No survivors
20CTP-10 HYCT	2181	2643	2352	2066	No survivors
30CTP- 0 HYCT	2526	2333	2325	2304	No survivors
Control	247	290	355	262	>90%
	Third-instar larvae in treated ´Tommy Atkins´ mangoes				
0CTP - 30 HYCT	2757	2221	2294	2072	No survivors
10CTP-20 HYCT	2496	2193	2152	2264	No survivors
20CTP-10 HYCT	2496	2502	2768	2664	No survivors
30CTP- 0 HYCT	2411	2749	2841	2856	No survivors
Control	254	303	314	288	>92%

CTP= Cooling time on platform (min). HYCT = Hydrocooling time (min).

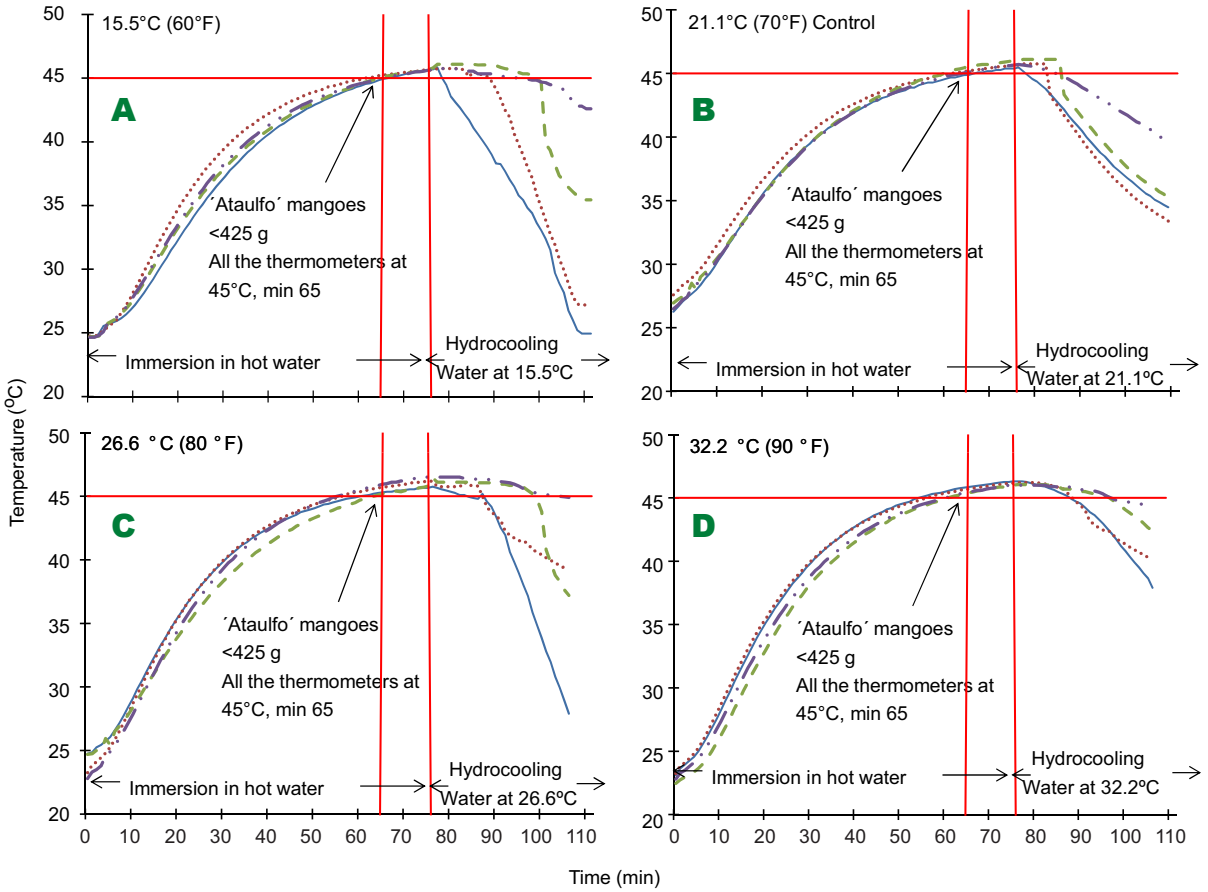

Fig.1. Heating curves of ´Ataulfo´ mangoes of <425g during hot water treatment and immediate hydrocooling.

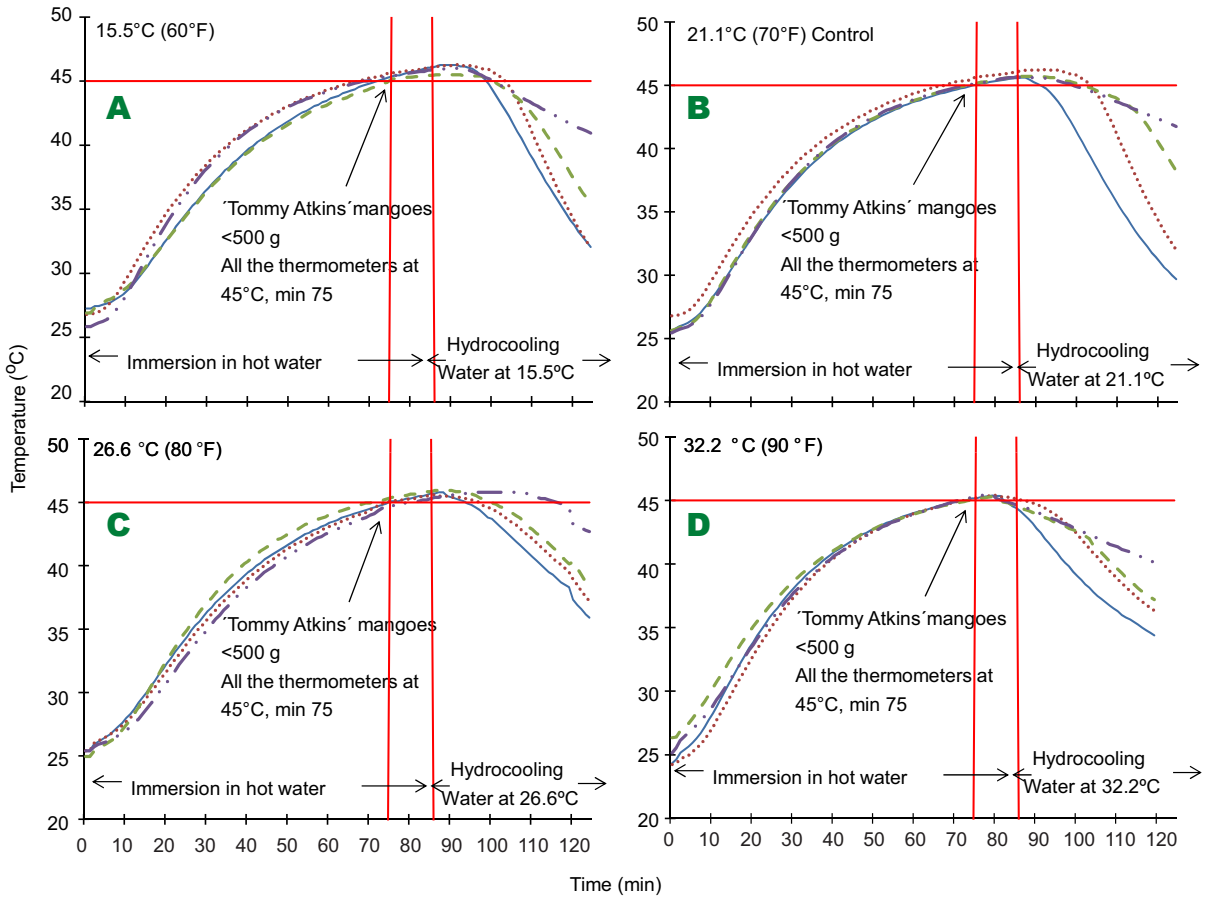

Fig. 2. Heating curves of 'Tommy Atkins' mangoes of 500 g during hot water treatment and immediate hydrocooling.

Table 2. Quality parameters of ´Ataulfo´ mangoes after hydrocooling for different times and at different temperatures

Cooling time	Temperature			
	15.5 °C (°60F)	21.1 °C (°70F) (Control)	26.6 °C (°80F)	32.2 °C (°90F)
		External color (°Hue)		
0CTP – 30HYCT	77.36±0.16 AB a	76.26±0.63 AB a	72.73±0.63 A a	55.26±4.90 A b
10CTP-20HYCT	74.81±0.49 B a	75.13±0.49 B a	72.65±0.35 A b	62.59±0.21 A c
20CTP-10HYCT	75.73±0.31 AB a	75.76±0.30 AB a	72.86±1.71 A a	59.14±1.23 A b
30CTP- 0HYCT	76.59±1.02 AB a	75.69±0.28 AB a	75.12±0.54 A a	60.88±0.56 A b
Control	78.62±1.08 A a	77.37±0.37 A a	73.95±0.30 A a	63.67±1.13 A b
		Pulp color (°Hue)		
0CTP – 30HYCT	83.35±1.33 A a	78.33±0.23 AB a	71.11±2.39 AB b	81.01±1.08 A a
10CTP-20HYCT	81.26±1.61 A a	77.43±0.33 B a	77.06±2.03 A a	81.95±0.84 A a
20CTP-10HYCT	77.11±1.24 A a	77.96±0.62 AB a	70.93±1.66 AB b	81.52±1.33 A a
30CTP- 0HYCT	81.92±1.29 A a	78.09±0.54 AB a	72.78±1.45 AB b	79.88±1.17 A a
Control	78.16±2.49 A a	79.41±0.25 A a	66.39±0.78 B b	81.05±1.35 A a
		Total soluble solids (°Brix)		
0CTP – 30HYCT	11.37±0.15 AB b	15.05±0.91 A a	13.58±0.55 A ab	13.62±0.66 A ab
10CTP-20HYCT	10.00±0.60 B b	11.80±0.33 A a	13.33±0.44 A a	12.88±0.35 A a
20CTP-10HYCT	13.43±1.07 A a	14.61±0.89 A a	13.18±0.38 A a	13.55±0.57 A a
30CTP- 0HYCT	12.27±0.11 AB a	13.00±0.19 A a	11.92±0.29 A a	13.00±0.63 A a
Control	10.83±0.73 AB c	14.68±0.57 A a	11.72±0.68 A bc	13.63±0.52 A ab
		Acidity (%)		
0CTP – 30HYCT	0.18±0.02 A a	0.09±0.01 A a	0.01±0.03 A a	0.09±0.03 A a
10CTP-20HYCT	0.12±0.01 B a	0.05±0.01 A a	0.11±0.04 A a	0.07±0.02 A a
20CTP-10HYCT	0.16±0.01 A a	0.11±0.04 A a	0.13±0.05 A a	0.09±0.03 A a
30CTP- 0HYCT	0.13±0.01 B a	0.11±0.01 A a	0.13±0.05 A a	0.10±0.03 A a
Control	0.06±0.01 C a	0.07±0.01 A a	0.12±0.05 A a	0.12±0.05 A a
		Firmness (kg-f)		
0CTP – 30HYCT	0.77±0.02 A a	0.66±0.01 BC a	0.78±0.07 A a	0.85±0.05 A a
10CTP-20HYCT	0.82±0.08 A a	0.59±0.03 C ab	0.31±0.10 B b	0.87±0.06 A a
20CTP-10HYCT	0.95±0.02 A a	0.79±0.03 A ab	0.51±0.13 AB b	0.76±0.08 A ab
30CTP- 0HYCT	0.85±0.06 A a	0.76±0.01 AB a	0.31±0.09 B b	0.83±0.06 A a
Control	0.95±0.09 A a	0.64±0.03 C ab	0.51±0.13 AB b	0.95±0.05 A a
		Weight loss (%)		
0CTP - 30HYCT	11.02±0.06 B b	11.08±0.07 B b	14.83±1.13 A a	14.36±0.35 A a
10CTP-20HYCT	11.03±0.04 B b	13.80±0.65 A ab	16.20±0.94 A a	17.04±1.55 A a
20CTP-10HYCT	12.65±0.32 A b	12.85±0.13 AB b	14.95±0.03 A a	14.32±0.98 A ab
30CTP- 0HYCT	11.97±0.45 AB b	13.19±0.75 A b	18.14±0.03 A a	16.67±0.30 A a
Control	12.07±0.13 AB b	5.93±0.06 C c	16.13±1.01A a	15.52±0.21 A a

CT*P*= Cooling time on platform (min). HYCT= Hydrocooling time (min). Values correspond to the Mean ± Standard Error. Values in rows with different capital letters indicate significant differences between cooling times. Values in columns with different lowercase letters indicate significant differences between temperatures.

weight loss values were lower in Tommy Atkins mangoes (Fig. 4E, F) compared to Ataulfo mangoes (Fig. 3E, F). Additionally, the measurements for Brix (Fig. 3C, Fig. 4C) and firmness (Fig. 3D, Fig. 4D) exhibited an inverse relationship, with Tommy Atkins mangoes showing lesser values.

Both Ataulfo and Tommy Atkins mangoes exhibited differences in cooling temperature and duration when immersed in water. However, the general trend observed between the two varieties remained consistent as illustrated in Fig. 5 (A-L). The lower values for acidity and weight loss in Tommy Atkins mangoes indicate a slower ripening process for this cultivar. Ataulfo mangoes exhibited a quicker ripening process compared to Tommy Atkins mangoes. However, both varieties typically reached the ripening stage suitable for consumption within a timeframe of 15 to 21 days.

Mango cv. ´Ataulfo´: Temperature ($P < 0.001$) and cooling time ($P = 0.016$), had a significant effect on external color (ºHue), while the interaction of temperature and cooling time was not significant ($P = 0.055$). There was a significant effect of temperature ($P < 0.001$), cooling time ($P = 0.010$), and the interaction between the two factors ($P = 0.001$) on pulp color. Sugar content, expressed as ºBrix, was significantly affected by temperature ($P < 0.001$), cooling time ($P = 0.006$), and the interaction of temperature and cooling time ($P = 0.005$). No significant effect of temperature ($P = 0.078$) or cooling time (P=0.441) was observed on acidity, but there was a significant effect of the interaction between the two factors ($P = 0.017$). Firmness (kgf m^{-2}) was significantly affected by temperature ($P < 0.001$), but not by cooling time ($P = 0.060$), and the interaction between the two factors was significant ($P = 0.002$). Finally, there was a significant effect of temperature ($P < 0.001$), cooling time ($P < 0.001$), and the interaction between the two factors ($P < 0.001$) on weight loss (%) (Table 2).

Mango cv. ´Tommy Atkins´: External color (ºHue) was significantly affected by temperature ($P < 0.001$), cooling time ($P = 0.010$), and the interaction of temperature and cooling time ($P < 0.001$). There was also a significant effect of temperature ($P < 0.001$), cooling time ($P < 0.001$), and the interaction between the two factors ($P < 0.001$) on pulp color. Sugar content, expressed as ºBrix, was also significantly affected by temperature ($P < 0.001$), cooling time ($P < 0.001$), and the interaction between the two factors ($P < 0.001$). Similarly, a significant effect of temperature ($P < 0.001$), cooling time ($P = 0.001$), and the interaction between the two factors ($P < 0.001$) was observed on acidity. Firmness (kgf m^{-2}) was also significantly affected by temperature ($P < 0.001$), cooling time ($P = 0.001$), and the interaction between the two factors ($P < 0.001$). Finally, there was a significant effect of temperature ($P < 0.001$), cooling time ($P < 0.001$), and the interaction between the two factors ($P < 0.001$) on weight loss (%) (Table 3).

Discussion

The three most important findings of this experiment are: 1) HWT is effective against infestation by *A. ludens*. 2) The fruit pulp reached 45°C by the end of the HWT, as demonstrated by the heating curves, causing larval mortality. 3) The fruits remained at 45°C for 8 to 10 min. 4) HYC does not compromise the hydrothermal treatment and minimize the impact on fruit quality. 5) HYC at 15.5°C tends to delay the ripening of both mango varieties, ´Ataulfo´ and ´Tommy Atkins´.

The "Treatment Manual Schedules" (USDA/MRP/APHIS/PPQ, 2013) and the "Work Plan for the Treatment

Table 3. Quality parameters of ´Tommy Atkins´ mangoes after hydrocooling for different times and at different temperatures

Cooling time	Temperature			
	15.5°C (°60F)	21.1°C (°70F) (Control)	26.6°C (°80F)	32.2°C (°90F)
		External color (°Hue)		
0CTP - 30HYCT	59.20±4.88 B a	58.97±2.14 A a	61.99±1.13 A a	42.03±3.15 B b
10CTP-20HYCT	77.88±1.01 A a	66.35±6.62 A ab	58.27±1.47 A bc	41.77±6.27 B c
20CTP-10HYCT	61.74±2.18 B a	57.82±2.05 A a	62.43±0.98 A a	60.16±1.71 A a
30CTP- 0HYCT	65.67±1.38 B a	62.99±1.79 A a	60.83±1.27 A a	59.46±2.81 A a
Control	54.81±2.64 B ab	51.97±2.56 A b	63.42±3.08 A a	58.83±1.64 A ab
		Pulp color (°Hue)		
0CTP - 30HYCT	78.94±0.42 C a	66.68±1.28 BC b	63.53±0.30 B c	62.93±0.52 B c
10CTP-20HYCT	77.88±1.01 C a	60.43±0.58 C b	61.25±0.78 B b	60.99±0.99 BC b
20CTP-10HYCT	83.46±1.13 AB a	81.23±0.82 A a	64.60±0.32 B b	60.84±0.84 BC c
30CTP- 0HYCT	82.75±0.94 B a	70.89±2.65 B b	61.74±0.47 B c	58.16±0.97 C c
Control	86.94±0.83 A a	61.02±1.46 C d	78.17±2.49 A b	68.97±0.41 A c
		Total soluble solids (°Brix)		
0CTP - 30HYCT	12.40±0.32 AB a	11.92±0.37 A ab	11.53±0.21 C ab	10.97±0.42 B b
10CTP-20HYCT	13.28±0.27 A a	11.30±0.19 A b	13.43±0.26 A a	13.65±0.16 A a
20CTP-10HYCT	13.15±0.33 A a	12.32±0.21 A a	12.37±0.23 BC a	10.72±0.21 B b
30CTP- 0HYCT	11.53±0.34 B b	11.93±0.31 A b	12.73±0.34 AB ab	13.48±0.53 A a
Control	13.05±0.38 A b	11.98±0.15 A b	12.35±0.21 BC b	14.00±0.38 A a
		Acidity (%)		
0CTP - 30HYCT	0.19±0.01 B bc	0.24±0.01 B b	0.39±0.02 AB a	0.16±0.02 B c
10CTP-20HYCT	0.18±0.01 B a	0.24±0.02 B a	0.22±0.03 C a	0.18±0.01 AB a
20CTP-10HYCT	0.29±0.03 A a	0.21±0.01 B a	0.22±0.03 C a	0.37±0.09 A a
30CTP- 0HYCT	0.24±0.02 AB ab	0.21±0.02 B b	0.29±0.01 BC ab	0.32±0.04 AB a
Control	0.21±0.02 AB b	0.39±0.02 A a	0.41±0.04 A a	0.20±0.01 AB b
		Firmness (kg-f)		
0CTP - 30HYCT	1.58±0.11 B a	1.66±0.26 AB a	1.80±0.03 A a	0.83±0.05 B b
10CTP-20HYCT	1.74±0.09 AB a	1.16±0.13 AB b	1.18±0.01 C b	0.97±0.11 AB b
20CTP-10HYCT	1.40±0.06 B a	1.41±0.12 AB a	1.39±0.04 B a	1.22±0.12 A a
30CTP- 0HYCT	1.35±0.04 B a	1.09±0.19 B ab	1.32±0.06 BC ab	0.91±0.05 AB b
Control	2.17±0.25 A a	1.86±0.11 A a	1.16±0.03 C b	0.70±0.03 B b
		Weight loss (%)		
0CTP - 30HYCT	5.28±0.02 B b	4.63±0.24 C c	6.03±0.18 A a	4.45±0.08 C c
10CTP-20HYCT	5.44±0.09 B bc	7.25±0.32 A a	5.79±0.24 A b	4.77±0.15 BC c
20CTP-10HYCT	4.79±0.14 C b	6.87±0.04 A a	4.87±0.07 B b	4.93±0.02 AB b
30CTP- 0HYCT	5.98±0.09 A b	7.11±0.04 A a	4.88±0.01 B d	5.17±0.05 A c
Control	5.17±0.03 B ab	5.50±0.06 B a	4.98±0.20 B bc	4.64±0.05 BC c

CT*P*= Cooling time on platform (min). HYCT= Hydrocooling time (min). Values correspond to the Mean ± Standard Error. Values in rows with different capital letters indicate significant differences between cooling times. Values in columns with different lowercase letters indicate significant differences between temperatures.

and Certification of Mexican Mangoes" (USDA/SAGARPA, 2020) established that: The temperature at the beginning of the hydrothermal treatment (HWT) must be >46.1, between 46.5 to 47.8 °C, mango pulp reached and at the end should be at least 45 °C, HYC temperature 21°C. Shellie and Mangan (2002b) mention that, after HWT, fruits cooling in plateform maintained the temperature of 45 °C during 8 to 10 min and this time extend the thermal stress is necessary to ensure the larval mortality. In this period of 8 to 10 minutes that mangoes fruits remain at 45°C after the HWT is because once the mango containers are extracted from the hot water tank, they are positioned to allow excess water to drain effectively. Subsequently, the containers are immersed in cold water.

Add 10 min to the HWT to immediately apply HYC is based on the work by Shellie and Mangan (2002a, b) and Hernández *et al.* (2007). However, heating curves referred by Shellie and Mangan (2002a, b) indicated that at the end of treatment the maximum temperature in mangoes pulp never reached critical temperature (45°C) to kill larvae. Nevertheless, Hernández *et al.* (2017) indicaed that when the treatment is applied according to the "set points" described in the "Work Plan for the Treatment and Certification of Mexican Mangoes" (USDA/SAGARPA, 2020) as are established for each tank certified at the commercial or experimental level the mango pulp reach 45°C needed to fruit disinfestation . To ensure that the larvae are exposed to lethal temperatures for a sufficient duration, it is recommended that the temperature of the fruit be recorded during each heat treatment and subsequent cooling.

Hydrocooling is a method used to rapidly remove heat from mangoes by utilizing the thermal conductivity of water. Low temperature is commonly used to reduce the metabolism of fruits and vegetables and delay ripening (Ayele and Bayleyegn 2017). Mango fruits are usually stored above 13 °C to avoid chilling injury (Vithana *et al.*, 2018, Liu *et al.*, 2023) and cooled after hot water treatment by immersion in water at temperatures above 21.1 ºC (USDA-MRP-APHIS-PPQ, 2013).

This study showed that cooling the fruits in water at 15.5 ºC delayed ripening without compromising the effectiveness of the treatment, which successfully ensured the mortality of third-instar larvae of *A. ludens*. In a previous study, Luna-Esquivel *et al.* (2006) observed that ´Ataulfo´ mangoes exhibited a uniform yellow color after HWT and are more appealing to consumers. Color changes result from the heating rate (Shellie and Mangan, 2002a, 2002b) and the duration of the treatment (Osuna-Garcia *et al.*, 2002) but do not necessarily indicate accelerated maturation. Additionally, the content of soluble solids (sugars) increased with weight loss, resulting in sweeter mangoes. Moreno-Hernández *et al.* (2024) reported that in "Shelly" mango fruits, the application of HWT extended shelf life up to four weeks in cold storage associated to the increases the expression of genes that contribute to the preservation of the quality. Ndlela *et al.* (2022) indicted that quality parameters as fruit firmness, weight loss, and total soluble solids are not affected by time of hot water treatment.

In summary, our study demonstrates that HWT (46.4-47.0 ºC for 65 and 75 min) is enough to ensure the mortality of third-instar *A. ludens* when the center of the mangoes, in this case cvs. ´Ataulfo´ of <425 g and ´Tommy Atkins´ of 500g, reaches 45 °C**.** And the HYC applied immediately after HWT not compromise the efficacy. In addition, hydrocooling at a temperature of 15.5°C tended to favor a delay in ripening in both mango varieties. Using HYC immediately after HWT ensures a linear and continuous process for the phytosanitary treatment and packaging of mangoes. Otherwise, the mangoes would need to wait on the platform to cool before continuing with packaging.

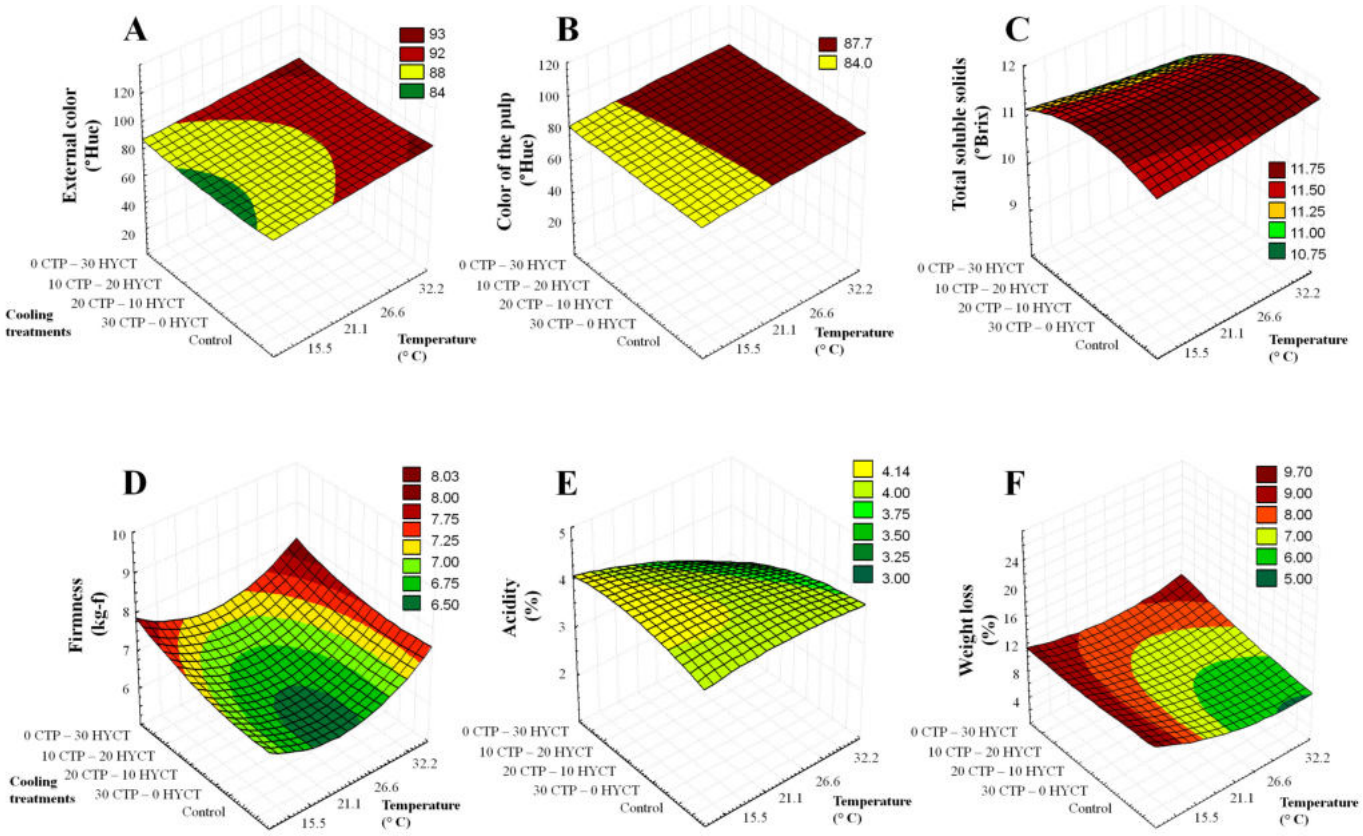

Fig. 3. Surface plots for quality parameters of 'Ataúlfo' mangoes of <425g during hot water treatment and immediate hydrocooling.

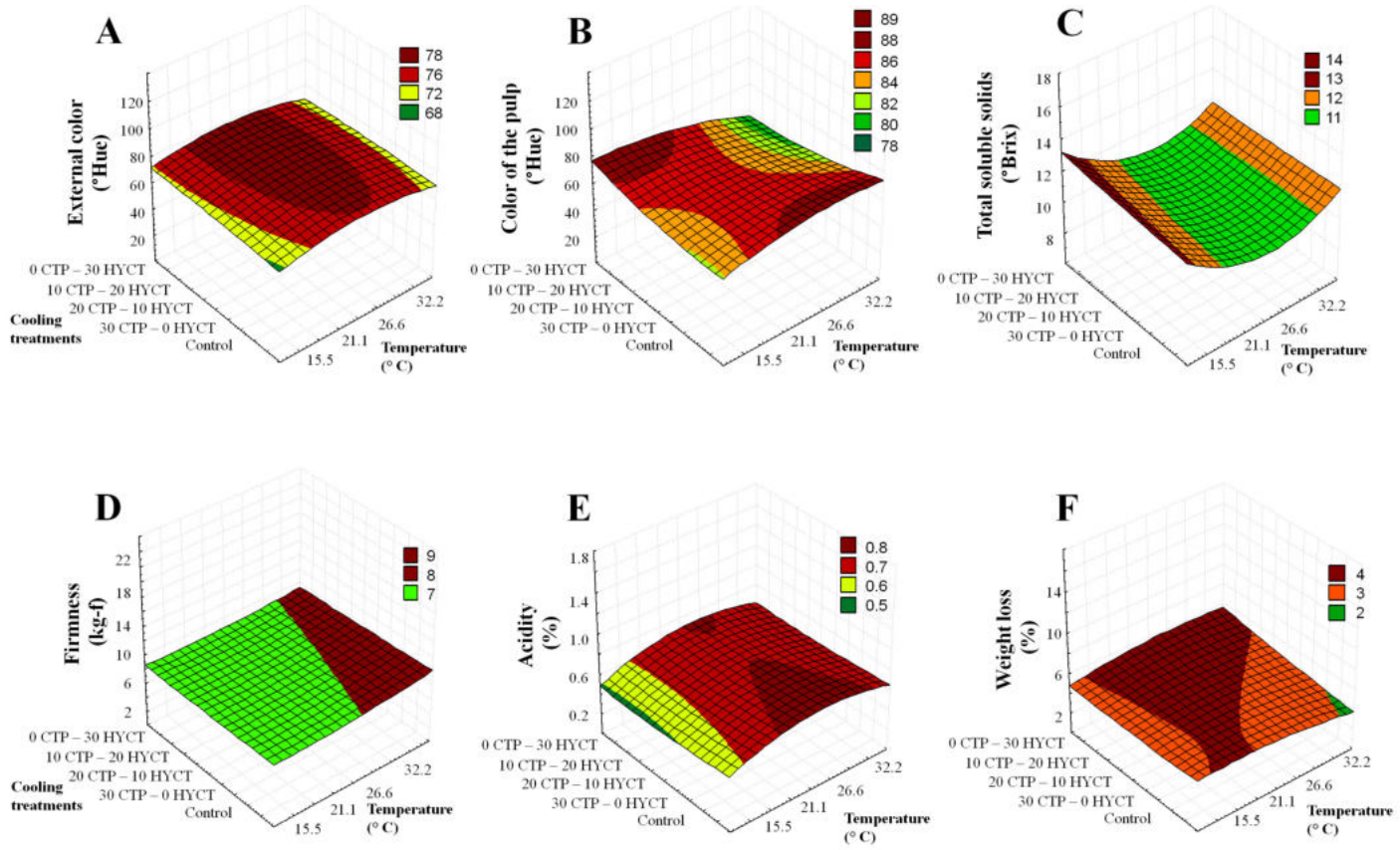

Fig. 4. Surface plots for quality parameters of 'Tommy Atkins' mangoes of <425 g during hot water treatment and immediate hydrocooling.

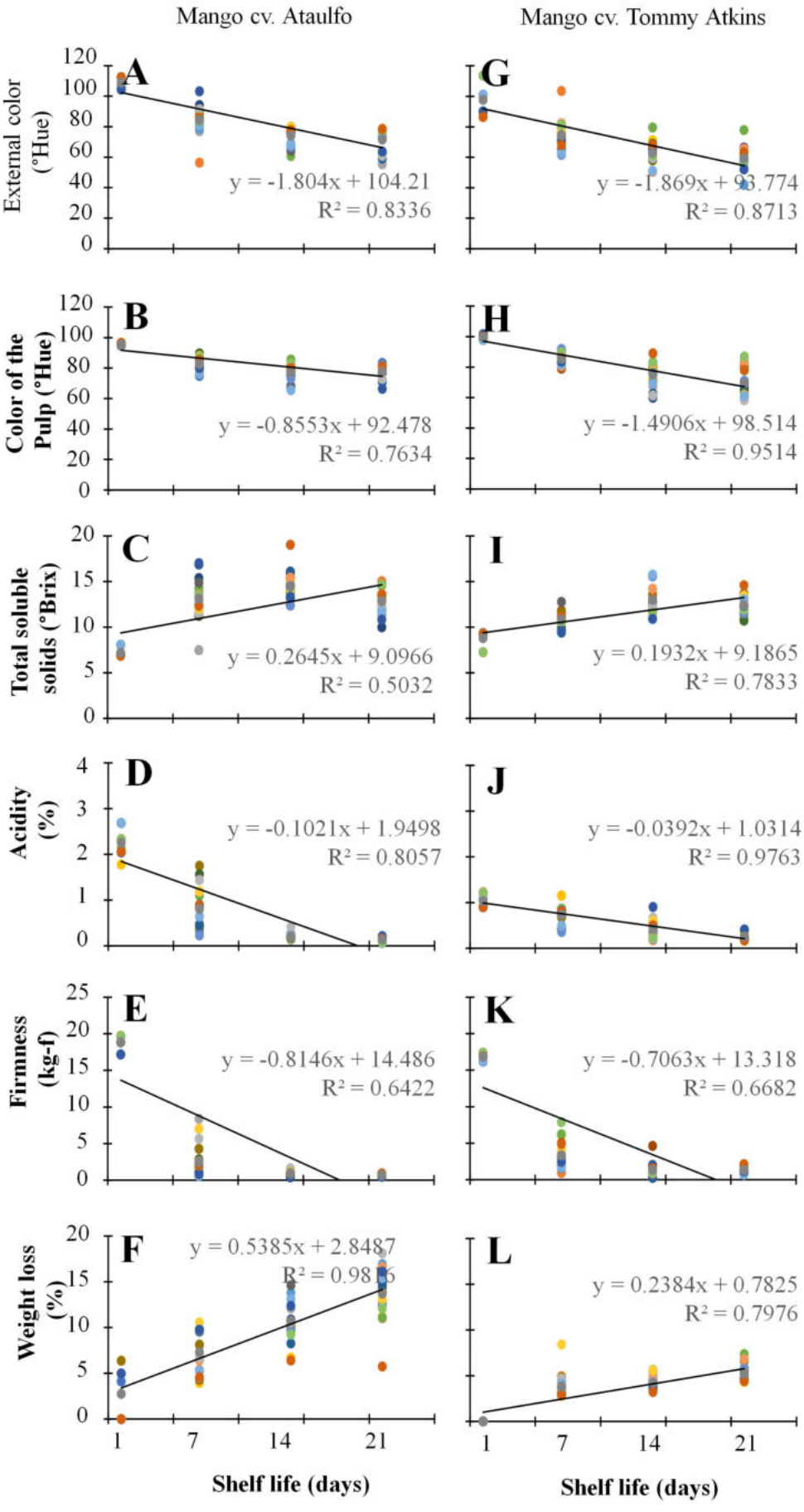

Fig. 5. Linear trend for quality parameters of 'Ataulfo' and 'Tommy Atkins' mangoes.

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