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Hydrocooling and forced-air cooling of litchi fruits (*Litchi sinensis* Sonn.) cv. Brewster: Quality and histological changes

Hidroenfriamiento y enfriamiento por aire forzado de frutos de litchi (*Litchi sinensis* Sonn.) cv. Brewster: Calidad y cambios histológicos

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Abstract

The effects of forced-air cooling and hydrocooling on the quality and storage life of litchi fruits, cv. Brewster, were evaluated. For this, freshly harvested, quality-graded fruits were cooled using equipment specially designed and built for each method; subsequently, they were stored at 7 ºC (95 % RH) and periodically assessed for 28 days. Although equipment functioning was optimal (i.e. short cooling times, uniform temperatures of 0.3–1.5 ºC inside the chambers, RH > 95 % during forced-air operations), there were no benefits to fruit quality except for lower values of weight loss (WL) in forced-air treated litchi. Chilling injury (CI) eventually appeared in hydrocooled fruits, and its early symptoms (browning and extreme desiccation) were characterized using both optical (OM) and scanning electron microscopy (SEM). CI symptoms, however, became noticeably reduced when Xtend® bags were used to create a modified atmosphere during storage. Thus, in order to limit CI and preserve fruit quality, forced-air cooling (air > 1.0 ºC) should be used instead of hydrocooling, along with modified atmosphere packaging (MAP) – especially for long-term storage. Finally, the unexpected development of CI in hydrocooled litchi indicates that this treatment is not always beneficial for tropical fruits, which are quite sensitive to low temperatures in general.

Keywords: Browning, chilling injury, dehydration, histology, MAP, modified atmosphere packaging.

Resumen

Se evaluó el efecto del hidroenfriamiento y del enfriamiento con aire forzado en la conservación de la calidad de litchi cv. Brewster. Se aplicó el enfriamiento a frutos recién cosechados y clasificados por calidad utilizando equipos diseñados y construidos exprofeso para cada método. Los frutos se almacenaron a 7 ºC (95 % HR) y la calidad se evaluó periódicamente durante 28 días. Aunque el funcionamiento del equipo fue óptimo (tiempos de enfriamiento cortos, temperaturas uniformes de 0.3–1.5 ºC dentro de las cámaras, HR > 95 % durante las operaciones de aire forzado), no se observó un efecto benéfico de ninguno de los dos métodos, excepto en la pérdida de peso que fue menor en los frutos enfriados con aire forzado. Más aún, se encontraron evidencias de daño por frío (DF) en los frutos hidroenfriados, y los síntomas tempranos de esta fisiopatía (pardeamiento y deshidratación severa) se caracterizaron por microscopía óptica (MO) y electrónica de barrido (MEB). Sin embargo, el DF se redujo notablemente cuando se utilizaron bolsas Xtend® para crear una atmósfera modificada durante el almacenamiento. Se concluyó que para limitar el DF y preservar la calidad de la fruta, se recomienda el enfriamiento con aire y no el hidroenfriamiento, con una temperatura del medio enfriante arriba de 1.0 ºC. Además, se recomienda el envasado con atmósfera modificada (MAP), especialmente si se estima que el almacenamiento del cv. Brewster será prolongado. Finalmente, el desarrollo inesperado de DF en litchi hidroenfriado indica que este tratamiento no siempre es beneficioso para las frutas tropicales, que son bastante sensibles a las bajas temperaturas.

Palabras clave: pardeamiento, daño por frío, deshidratación, histología, MAP, envasado en atmósfera modificada.

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1 Introduction

Litchi sinensis Sonn. is a species in the Sapindaceae family that grows in tropical and subtropical climates. Today it is found in many regions despite being native to Southeast China, with six countries (China, Taiwan, Thailand, India, Madagascar, and South Africa) leading the world in total annual production of this fruit. As a result, the litchi market size is estimated at USD 7.10 billion in 2024 and is expected to reach USD 9.27 billion by 2029 (Mordor, 2024).

The fruit was introduced to Mexico over a century ago, specifically to Sinaloa – a state in the northwest region of the country. From there, it was brought to other areas in the east (e.g. the state of Veracruz), central-east (Puebla), and south (Oaxaca), gaining greater commercial significance during the process (Schwentesius and Gómez, 2001). For instance, in 2023 alone, Mexican national production reached 26 thousand tons – roughly equivalent to 520 million pesos or 28 million USD (SAGARPA-SIAP, 2023).

Litchi is a non-climacteric, ovoid drupe that is typically 2–3.5 cm in diameter. Externally reddish to reddish-brown in color, its growth and development usually span 80–112 days. Anatomically, the fruit is composed of a single seed wrapped in aril tissue. This tissue (the soft edible portion of the fruit) is in turn surrounded by the fruit pericarp or peel. The pericarp itself consists of three layers: the exocarp, mesocarp, and endocarp (Wang *et al*., 2017)

The exocarp, when immature, is histologically composed of an external epiderm and two hypodermic layers. The external epiderm consists of a series of small, cube-like cells with radially thickened walls which are covered by a thick cuticle containing phenols. Formation of sclerenchymatous tissue begins just below the epiderm and is essentially complete 62 days after anthesis. This sclerenchymatous tissue is distributed into two or three discontinuous layers, separated by very noticeable protuberances in the pericarp (Nacif *et al*., 2001).

The mesocarp, on the other hand, consists of anthocyanin-producing cells in the exterior and a "spongy" parenchymatic tissue on the inner side (the one facing the fruit interior). The anthocyanin is a bioactive compound which could potentially provide many health benefits (Loan *et al*., 2024).

The endocarp is histologically simpler still, containing very small non-lignified cells with thin walls. Vascular tissue can be found throughout the pericarpial region, though it is most abundant in the internal mesocarp (Nacif *et al*., 2001; Fahima *et al*., 2019).

Endocarp cells separate from the aril when the fruit matures. The aril (the soft edible portion) is greatly appreciated for its perceived "freshness", excellent flavor, juicy texture, whitish color and can be found covering the seed completely (Nacif *et al*., 2001; Fahima *et al*., 2019).

In Brewster litchi fruit the seed is large, which gives its name as normal seeded (Wang *et al*., 2017). Anzurez-Valdez *et al*. (2022) have reported that the use of ethanol extract of litchi seeds has effects on aluminum corrosion, since it behaves as an anodic inhibitor by the formation of a protective corrosion products layer which blocked further corrosion of metal by electrolyte.

Apart from the cuticle that covers the outer surface of the exocarp, a second layer of cuticle is found covering the inner surface or endocarp. Both are important barriers that prevent water loss from the aril to the surrounding environment (Riederer *et al*., 2015). This is important as litchi is quite perishable after harvest – quickly dehydrating and losing weight at the usual field temperatures of most tropical and subtropical climates. The presence of small cracks in the fruit pericarp is usually the reason for such a high susceptibility to dehydration (Huang *et al*., 2004). These cracks are formed during the early phases of fruit development, in a period of rapid expansion of the aril flesh. This results in a build-up of pressure against the pericarp, which by this time has stopped growing (Kumar *et al*., 2017). Thus, the distribution and commercialization of litchi in both domestic and international markets suffer significantly.

A second problem, common to fruits of tropical origin, is its notorious sensitivity to refrigerated storage. This usually leads to chilling injury (CI) which, together with dehydration, results in a loss of the original red color – making it hard to distinguish between the two physiopathies (Holcroft *et al*., 2005; Kumar *et al*., 2017). Dehydration, however, causes the epicarp to thin and change color to a pale yellow or light brown, whereas CI leads to a more intense browning of the pericarp, which is often accompanied by localized darkening and hydrosis (i.e. the formation of water-soaked tissue) (Holcroft *et al*., 2005; Kumar *et al*., 2017). Dehydration-associated browning begins immediately after harvest and may affect the whole fruit after just three days at room temperature (and 65–70 % RH; Huang *et al*., 2004). On the other hand, CI-browning and its associated symptoms usually appear after litchis have been kept at sub-optimal temperatures during cold storage – increasing in visibility only after transfer to higher, non-chilling temperatures (Jiang *et al*., 2003). According to Huang *et al*. (2024), polyphenol oxidase and peroxidase are two enzymes likely to be involved during the early stages of browning in general that affect the color and marketability of horticultural products as occurs in peach slices as reported by Garcia-Moreira *et al*. (2024).

Hu (2004) reports that litchi is sensitive to CI

when stored at temperatures below 3 ºC. At 0 ºC, for instance, the pericarp gradually darkens, and both CIE L*a*b* (color space, mathematically defined by the Commission Internationale de l´Éclairage) and the concentration of certain compounds (e.g. anthocyanins) decrease. As CI expands, however, dehydration occurs and begins to affect the structure of pericarp cells, leading to an increase in both the permeability of membranes and of cellular pH (Liu *et al*., 2011). This results in a rapid deterioration of fruit quality when litchis are returned to room temperature. A recent study by our research group also supports this general trend (unpublished data). In this case, weight loss (WL) and localized darkening of the pericarp were indeed greatest in fruits kept at 1 and 3 ºC compared with those stored at 7 ºC (i.e. the "safe temperature" recommended for cv. Brewster).

Thus, it is recommended that litchi be cooled before refrigerated storage using either forced-air cooling or hydrocooling (the purpose of cooling is, after all, to eliminate field heat quickly in order to preserve fruit quality during transport and storage) (Liang *et al*., 2013; Oliveira *et al*., 2015; Mahajan *et al*., 2019). The two aforementioned methods, however, are not equal in terms of costs (forced-air cooling is generally more expensive; Bagshaw *et al*., 2000), cooling times (3–4 h using forced-air cooling vs. 15–20 min with hydrocooling; Thompson *et al*., 2007; Kumar and Dutt, 2018), or microbiological concerns (e.g. if not adequately disinfected, residual water may cause infections in hydrocooled litchi (Brosnan and Sun, 2001). Additionally, their ability to preserve fruit quality, as well as their effectiveness and differences in this regard, have not been adequately studied in litchi.

Generally, among the most widely used methods for maintaining fruit quality and prolonging shelf life are modified atmospheres (Ali *et al*., 2019). These can be generated by placing fruit inside special-purpose containers or bags (e.g. Xtend® bags) and are typically used in combination with refrigeration. For instance, it has been reported that the use of Xtend® bags limits both CI and the extent of pericarp browning in litchi (Jiang *et al*., 1999; Porat *et al*., 2004; Ali *et al*., 2019).

The aim of this work then, was to evaluate the effect of forced-air cooling and hydrocooling on litchi quality and postharvest life. For this purpose, *Litchi sinensis* Sonn. cv. Brewster was selected as it is the dominant variety among most commercial plantations in Mexico. Early symptoms of CI (browning and severe desiccation) were also characterized using both optical (OM) and scanning electron microscopy (SEM) as current studies mostly describe macroscopic symptoms in litchi. The effectiveness of Modified Atmosphere Packaging (MAP) as an ancillary method to limit the presence of CI was additionally tested. It is hoped that with these results, the most suitable method(s) for the large-scale cooling and storage of litchi will be selected.

2 Materials and methods

2.1 Cooling equipment

As none of the litchi packing facilities visited in Mexico had the necessary equipment for either forced-air cooling or hydrocooling operations, existing mobile equipment from a previous study (Chatelain-Mercado *et al*., 2008) was used instead. One of these machines was designed to operate with forced air, the other with disinfected water (applied by immersion, i.e. hydrocooling). A more detailed explanation of equipment design and functioning can be found in the aforementioned study.

2.2 In-field application of forced-air cooling and hydrocooling

Litchi fruit (*Litchi sinensis* Sonn.) cv. Brewster from two commercial orchards located in the Mexican states of Oaxaca (municipality of Tuxtepec) and Puebla (municipality of Venustiano Carranza) was used as biological material. The fruit from Oaxaca was harvested at ¾ maturity (bright, pink-red epicarp) as it is generally exported, whereas fruit from Puebla was harvested at full maturity (uniformly red) since it is intended for domestic markets. The use of two maturity stages also provides valuable information about the sensitivity of each to CI.

Immediately afterward, the fruit was cleaned (leaves and small branches were removed), screened (according to size, uniformity of color, and absence of defects), and quality-graded (using the appropriate standard for their intended market). From this second pool of graded pre-screened fruit, random samples were taken, which constituted the final set of litchis to be used during the two types of cooling operations.

For air cooling, the fruit was placed directly inside the forced air chamber of the corresponding machine; for hydrocooling, they were first submerged in water at room temperature. This immersion was intended to condition recently arrived (warm) produce to further drops in temperature as immediate contact with cold water can cause internal atmospheres to contract, allowing liquid to penetrate the fruit. Even though the water used here was already potable in theory, it was decided to further sanitize it with chlorine (0.1 g L^{-1} of sodium hypochlorite, NaClO) in order to guarantee microbiological safety.

The operating time of both machines (the forced-air and hydrocooling equipment) was strictly the length necessary to reach 7/8 cooling – that is, 7/8 of the difference between the initial temperature of

the fruit (essentially the field temperature) and the temperature of the cooling medium (in this case, air or water maintained at 0.3–1.5 ºC) (Thompson *et al*., 2007).

2.3 Fruit handling after cooling, experimental design, and quality assessment

After cooling, the fruit was packaged inside vented polylactic "clamshell" containers (450 g capacity, 18-26 litchis depending on fruit size) and maintained at 7 ºC in the refrigerated chambers of the packing facility, awaiting transportation. A refrigerated vehicle at the same temperature was then used to transport the fruit back to the lab for storage at 7 ± 1 ºC and 95 % RH for 3–4 weeks. All throughout handling, special care was taken so as not to break the cold chain.

2.3.1 Experimental design

An experimental unit consisted of a single clamshell container with either 30 (forced-air treated) or 20 (water treated) fruit. Controls consisted of this same two groups without any cooling treatment. There were three repetitions per treatment (including the control), and a randomized experimental design was followed.

2.3.2 Measured variables

At regular intervals during storage, triplicate samples were taken from each treatment (cooling method) to assess weight loss (WL), damage index (DI), quantity of marketable fruit (MF), total soluble solids (TSS), and titratable acidity (TA). WL was measured on an Ohaus digital scale and reported as the percentage of lost weight relative to the initial weight of the fruit. DI scores were calculated using the following four-point scale: 0 = fruits with no damage (i.e. no browning or black areas) on the surface; 1 = fruits with ≤ 10 % surface damage; 2 = fruits with ≥ 10 % surface damage, but still less than 20 %; and 3 = fruits with > 20 % surface damage. This scale was based on Codex Stan 196-1995 (Quality Standard for Litchi, Codex Alimentarius Commission), with some modifications. DI was then calculated using the equation below:

$$\text{Damage index (D}I\text{)} = [(\text{No.fruits grade 0 X 0}) + (\text{No.fruits grade 1 X 1}) + (\text{No.fruits grade 2 X 2}) + (\text{No.fruits grade 3 X 3})]/(\text{Total number of fruits evaluated})$$

In essence, this value (MF) was obtained by adding litchis in grades 0, 1, and 2 (two being the maximum score possible for fruit still considered to be "marketable") and calculating their percentage relative to the total number of fruits per treatment. As a reference, traders will still accept produce if non-MF are ≤ 10 % of the total fruit (personal communication, 2016). TSS, on the other hand, were determined using a field refractometer and a few drops of the juice extracted from an experimental unit (i.e. 20–30 litchis); the results were then reported as percentages. Finally, TA was quantified via acid-base neutralization using 0.1N sodium hydroxide and phenolphthalein as an indicator dye; in this case, the results were expressed as percentages of malic acid (the dominant organic acid in litchi pulp) (AOAC, 2019). The TSS/TA ratio was then calculated from these last two variables.

2.3.3 Statistical analysis

An analysis of variance and comparison of means (Fisher's LSD test) were performed using Number Cruncher Statistical Systems (NCSS, 2016).

2.4 Histological characterization of browning and desiccation

To examine these two early symptoms of CI in litchi (as brought about by cooling and sub-optimal refrigeration), a histological study was conducted on a separate group of fruits from Tuxtepec, Oaxaca. These were harvested at full maturity (ripeness) and selected according to size, color, and the absence of defects. They were then divided into four groups consisting of 30 fruits each, and placed inside vented, polylactic clamshell containers except for those in group 4, which were placed inside a single Xtend® bags instead. The four groups were then treated as follows:

Group 1: Freshly harvested fruits (serving as the first reference).

Group 2: Fruits stored at 25 ºC (RH 45 %) for 3, 6, and 10 days (serving as the second reference). Ten days is the maximum storage period for litchi at this temperature.

Group 3: Fruits stored at 3 ºC (RH 65 %) for 3, 6, and 10 days. This temperature is sub-optimal and found to cause extreme desiccation in cv. Brewster (unpublished data).

Group 4: Hydrocooled fruits stored inside Xtend® film bags (polyolefin-based, with low water vapor transmission rates [WVTR]; Johnson Matthey, United Kingdom; StePac, Tefen, Israel) and maintained at 3 ºC (RH 65 %) for 3, 6, and 10 days. In the present study, this method of cooling was found to promote desiccation, while the Xtend® film bags can decrease dehydration and prevent the excess of moisture and the subsequent microbial growth (Ali *et al*., 2019).

After treatment, the fruits were randomly sampled from each of these four groups and histological preparations of the pericarp and pulp were prepared for imaging in both optical and scanning electron microscopes.

For the former (optical micoscope), permanent slides were prepared using tissue samples fixed in FAA (formaldehyde, acetic acid, ethanol). These were dehydrated in a series of terbutyl alcohol mixtures of increasing concentration and transferred to xylol-paraffin paraffin (50/50 v/v) and paraffin (100 %) at 60 ºC for 24 h. The samples were then mounted into molds containing paraffin and cut into 10–12 μm sections with a rotatory microtome (American Optical 820). The paraffin was removed by soaking in xylol, then ethanol (solutions of decreasing concentration), and the resulting slides colored by immersion in aqueous safranin (1 % w/v for 12 h). These were then washed in ethanol (solutions of increasing concentration) and transferred to an ethanolic solution of Fast Green for three seconds. The slides were given one final rinse in both ethanol and xylol before being mounted in synthetic resin for observation (Gray, 1964).

For scanning electron microscopy (SEM) imaging, the procedure followed was that of Bozzola and Rusell (1999) with certain modifications: Briefly, pericarp samples were first fixed in 6 % glutaraldehyde, washed 3 times with 200 mM phosphate buffer (pH 7.2), and further fixed and stained using 2 % osmium tetroxide. Next, they were dehydrated in both ethanol (solutions of 30–100 % v/v) and liquid carbon dioxide (Samdri-780B critical point dryer) before being coated in a thin layer (10 nm) of gold. The cut surfaces were then observed under a Zeiss DMS-960A scanning electron microscope.

3 Results and discussion

3.1 *Efficiency of forced-air cooling and hydrocooling*

The temperature recordings in both coolers indicated only minor variations during these experiments (0.4–1.5 ºC for forced-air; 0.3–1.2 ºC for hydrocooling). Nevertheless, operating times varied greatly due to the nature of the processes themselves (4 hours for forced-air vs. 15 minutes for hydrocooling). During these periods, the temperature of the fruits decreased from 25 ºC to 4–5 ºC in both cases. These results, together with the operating data, are consistent with efficient equipment functioning.

3.2 *Effect of cooling on litchi quality and postharvest life*

Weight loss increased significantly with storage time as reported by Tsang and Furutani (2006) and was greatest in fruits from Oaxaca (Figure 1). This can partly be explained by their relatively small size (avg. of 17 g, compared to 25 g in litchis from Puebla) and state of maturity at harvest (¾ maturity vs. full maturity in litchis from Puebla). Smaller fruit, for instance, have greater surface-to-volume ratios and thus higher rates of evaporation, whereas less mature fruit probably have underdeveloped cuticles that offer less protection against dehydration (Riederer *et al*., 2015).

In this regard, temperature control is one of the key methods for slowing down quality loss in perishables; hence, both forced-air cooling and hydrocooling are expected to prolong the storage life of horticultural products (such as logan, rockmelons and sorrel) (Liu et al., 2021; Zainal *et al*., 2019; Ferreira Gomes *et al*., 2023). That WL was lower in forced-air treated litchi than in controls is therefore to be expected (Figure 1A and B); however, it was surprising to find higher values of WL in hydrocooled fruits from Oaxaca than in controls. In fact, the magnitude of WL in the former (and despite an optimal storage temperature of 7 ºC) strongly suggests the presence of CI. It is interesting to notice that the higher WL in hydrocooled fruit compared to the control occurs only after 21 days. However, the damage index was presented from 7 days of storage in hydrocooled fruit.

As briefly outlined in the introduction, a recent study by our research group determined WL to be greatest in fruit kept at 1 and 3 ºC (where CI symptoms occurred) compared with those stored at 7 ºC (where CI symptoms did not occur) (unpublished data using cv. Brewster). Thus, CI might also be responsible for WL in hydrocooled litchi, except that in this case the physiopathy was most likely induced by the faster temperature decline of this particular method of cooling.

Interestingly, epicarp damage was also greatest in hydrocooled fruits from Oaxaca during the first half of the storage period (Figure 1C). By comparison, a similar degree of damage (DI = 2.5; faster browning and emergence of dark areas in the epicarp) was only reached by forced-air treated litchi from Puebla until approximately day 21 (Figure 1D). Despite this, DI values close to the commercial acceptability limit of 2.0 were reached by all groups (i.e. forced air and hydrocooled fruit, irrespective of origin) after 21 days. There were, additionally, no significant differences between these values and those of controls. As a result, no real benefits to cooling were observed in litchi cv. Brewster – at least in terms of visible damage.

Likewise, there were no significant differences between the percentages of marketable fruit (MF) in litchi from Oaxaca during most of storage (Figure 1E). This applied to all treatments and also included controls. However, important declines did occur among pre-cooled fruits, especially towards the end (day 21). At that moment, the only group with a value of MF closer to the commercial acceptability limit of 90 % was the control (82 %) (Figure 1E).

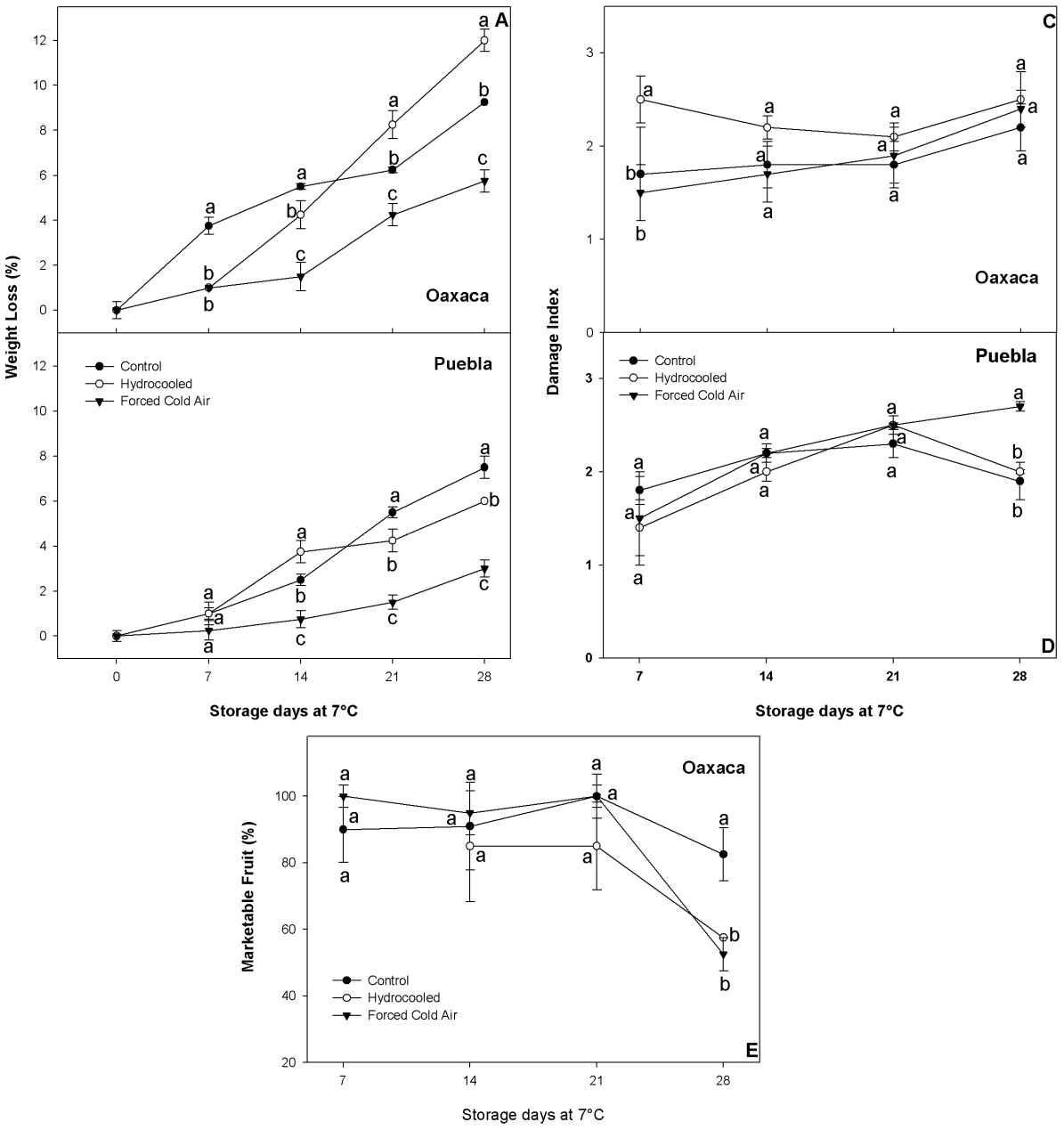

Figure 1. Quality assessment of pre-cooled litchi fruits from the states of Oaxaca and Puebla (Mexico). A, B: weight loss; C, D: damage index; E: percentage of marketable fruit. Data points represent the average of three repetitions ± SD. Different letters indicate significant differences between treatments (Fischer´s LSD test).

Similar results were obtained with fruits from Puebla (data not shown).

On the other hand, the initial TSS/TA ratios of litchi from both Oaxaca (35) and Puebla (79) were higher than those frequently used as harvest indices (Figure 2E and F). For instance, litchis in Australia must reach TSS/TA ratios of at least 30–40 if a good postharvest quality is to be ensured (Underhill and Wong, 1990). Similarly, litchis in South Africa must attain TSS:TA values of at least 30:1 when grown in temperate regions (Schoeman *et al*., 2005) and of 40:1 when cultivated in warmer climates (Cronje, 2008). In this experiment, lower initial TAs (0.56–0.23 %; Figure 2C and D) compensated for the presence of fewer soluble solids (TSS = approximately 19.6–18.1 %; Figure 2A and B), resulting in favorable TSS/TA ratios (Figure 2E and F). However, as storage time progressed, TSS/TA ratios increased to values greater than 100 in litchis from Puebla while changing little in fruits from Oaxaca. Despite this, any value between 100 and 120 is still considered acceptable in terms of taste (unpublished data using cv. Brewster).

As before, cooling had no noticeable effect in any of the aforementioned parameters. However, hydrocooled litchi from Oaxaca (harvested at 3/4 maturity) experienced an abnormal increase in acidity

towards the end of storage – something that was not observed in hydrocooled fruits from Puebla (harvested at full maturity; see Figure 2C and D). This, again, suggests the presence of CI – though it is perplexing that the latter (i.e. fruits from Puebla) did not experience a similar change. Litchis are, however, more resistant to chilling injury when fully mature (Wang, 2010; Peng et. al., 2013; Jing *et al*., 2018), a fact that would account for this difference in behavior.

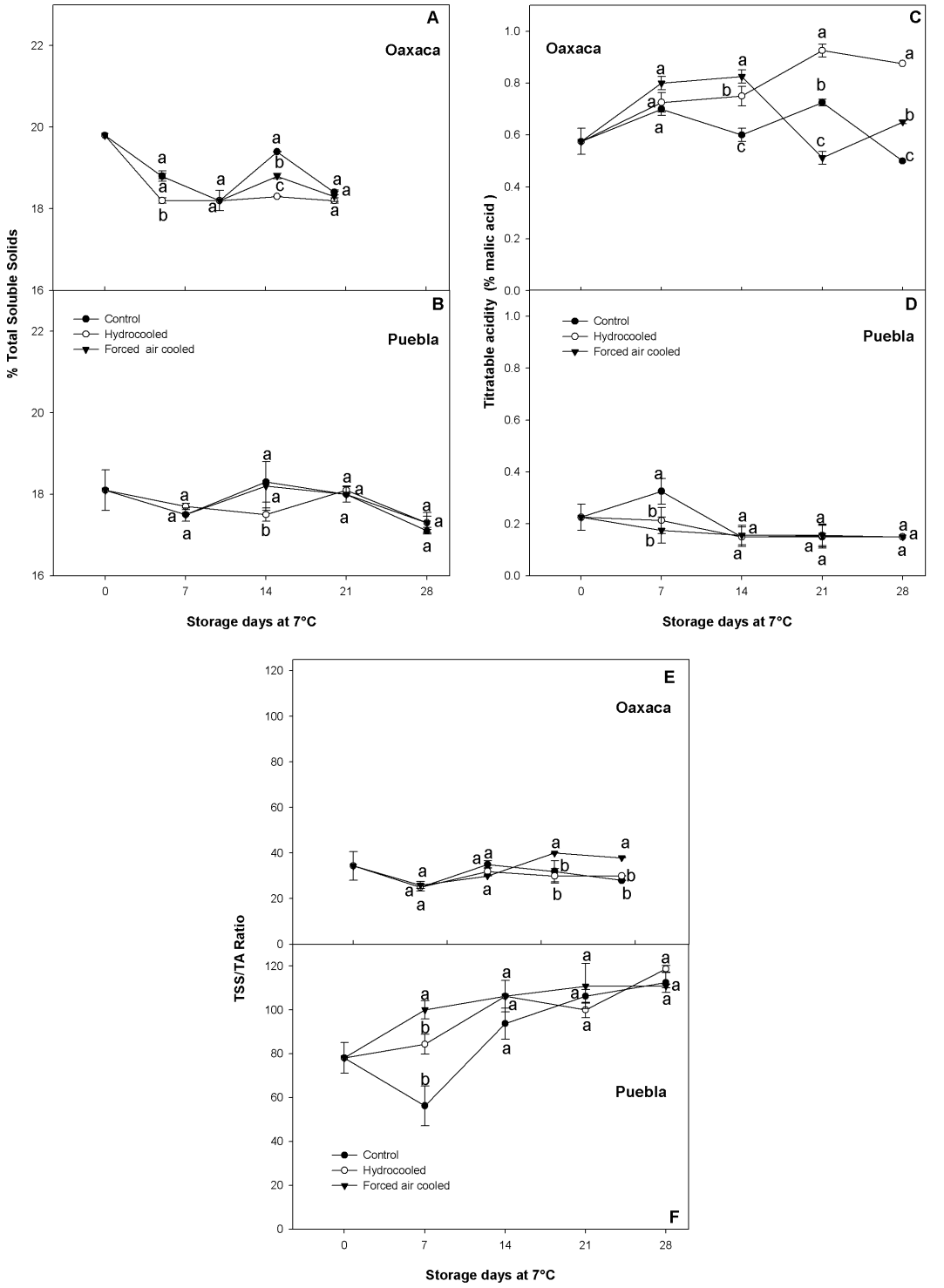

Figure 2. Juice quality attributes of pre-cooled litchi fruits from the states of Oaxaca and Puebla (Mexico). A, B: total soluble solids (TSS); C, D: titratable acidity (TA); E, F: TSS/TA ratio. Data points represent the average of three repetitions ± SD. Different letters indicate significant differences between treatments (Fischer´s LSD test).

Table 1. Characteristics of litchi fruits used for histological analyses.

Treatments	Size (cm)		TSS in juice (%)	*Pericarp color (% of total surface) (% of total surface)
	Length	Width		
Fresh fruits	3.64 ± 0.17	3.03 ± 0.19	15.5 ± 3.3	R 60, Y10, B 30
Fruits stored for 6 days at 25 ºC	3.43 ± 0.16	2.85 ± 0.16	22.3 ± 2.8	B 100
Fruits stored for 10 days at 3 ºC	3.40 ± 0.1	2.94 ± 0.16	16.9 ± 0.53	R 60, B 40
Hydrocooled fruits in Xtend bags stored for 10 days at 3 ºC.	3.44 ± 0.18	2.9 ± 0.12	16.72 ± 0.8	R 77, A 20, B 3

*R=red, Y=yellowish, B=brown

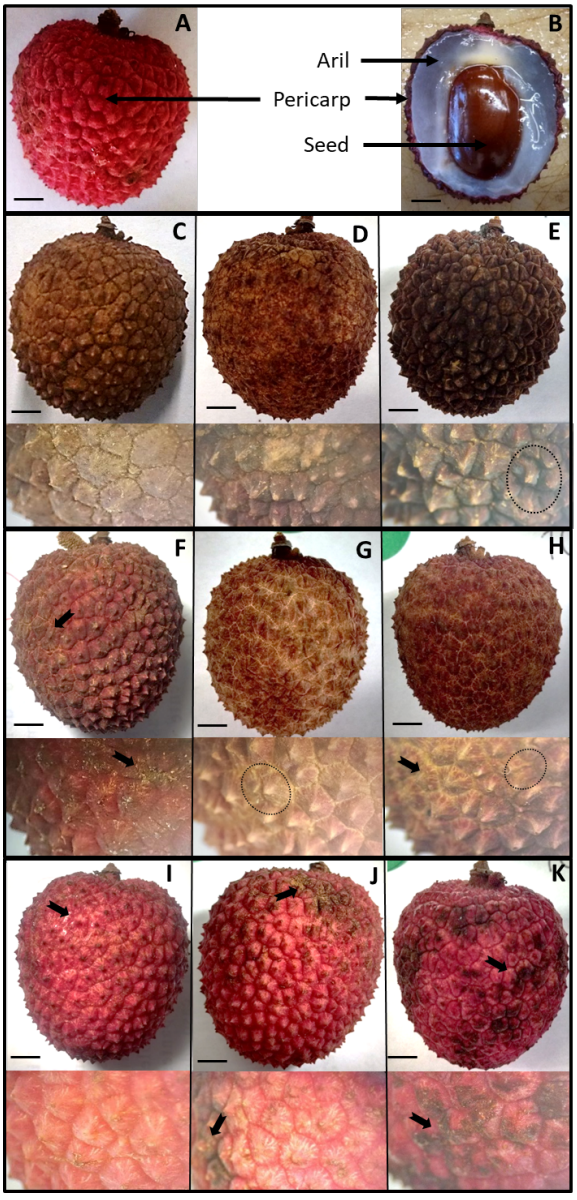

Figure 3. Dehydration and color change in the litchi pericarp as function of pre-storage and storage conditions. A: freshly harvested, ripe fruit; B: longitudinal section of whole fruit; C, D, and E: stored at 25 ^{o}C for 3, 6, and 10 d, respectively; F, G, and H: stored at 3 ^{o}C for 3, 6, and 10 d, respectively; I, J, and K: hydrocooled and MAP-stored at 3°C for 3, 6 and 10 d, respectively. Circles show location of micro-cracks, arrows damage by chilling injury. Bar = 52.5 mm.

Cooling, therefore, does not necessarily benefit litchi quality as it is so often reported in the literature (Sheng- pin, 2013). As evidenced by this study, only transpiration-associated WL was clearly and consistently reduced in forced-air treated fruit during most of storage. On the other hand, the biggest increases in WL, visible damage, and TA all occurred in hydrocooled litchi – symptoms strongly reminiscent of CI. As discussed previously, this physiopathy was most likely induced by the quick temperature decline of hydrocooling, thus explaining its absence from forced-air treated fruits.

3.3 Histological characterization of browning and desiccation

Pericarp browning and desiccation are common during the post-harvest deterioration of litchi and can begin immediately after harvest, resulting in fruit with an unfavorable appearance and quality (Devi et al., 2021; Huang *et al*., 2023). In such cases, water escapes the cuticle non-specifically (i.e. through generalized diffusion) or via micro-fractures that allow vapor to pass from the aril to the pericarp, and from there, to the exterior (Riederer *et al*., 2015; Fahima *et al*., 2019). WL in litchi is similarly associated with the presence of micro-fissures, which in this case, appear in the pericarp during the final stages of fruit development (Huang and Xu, 1983; Underhill and Simons, 1993; Jiang and Fu, 1999; Cronje, 2008). This not only results in greater oxidative stress and desiccation, but also in a higher propensity for pathogen invasion (Underhill and Critchley, 1992). Fissures are therefore present from the very beginning and persist regardless of the temperature and RH applied. What storage conditions can do, however, is limit the deterioration of the pericarp and pulp, thus minimizing WL and the loss of red color in the fruit exterior.

Minh *et al*. (2019) report that litchi fruits have shelf lives of 24–72 h under ordinary room conditions, and of ~20 days when stored at 2–5 ºC. Similarly, during the present study, desiccation and browning occurred after 3 days in litchi stored at 25 ºC (Figure 3C), with severe dark-browning emerging by day 10 (Figure 3E). This browning could be delayed a further 3–7 days (i.e. 6–10 days in total) if fruits were maintained at 3 ºC (Table 1, Figure 3G and H), and when additionally, hydrocooled and stored inside Xtend® bags (also at 3 ºC), their brilliant red color persisted (in this case up to 10 days; see Table 1 and Figures 3I, J, and K). The reason for this may lie in the relatively high RH created inside the polyolefin-based packaging – a result of normal fruit transpiration. After all, prior studies (Molla *et al*., 2017) have likewise reported higher L*C*h values as well as lower WL and decay at similar temperatures (5 ± 1 ºC), while passive MAPs (e.g. non-perforated polyethylene packaging used along with brown paper wrapping) have been demonstrated to increase both L*C*h values and firmness (as well as reduce browning) in litchis stored at 5 ± 1 ºC (Patel *et al*., 2016). Thus, polymeric films tend to decrease water loss along with some of its more visible effects (Kader, 1986).

Desiccation and browning have further been linked to changes in the anatomy of the pericarp and pulp (Underhill and Simons, 1993). These occur as a result of the activity of certain enzymes – namely polyphenol oxidase (PPO), peroxidase (POD), and laccase anthocyanin degradation enzyme (ADE). All three are also associated with the formation of micro-fissures on the litchi pericarp (Jiang *et al*., 1999; Zhang *et al*., 2005; Fang *et al*., 2015). Low temperatures, however, limit further deterioration and reduce the loss of anthocyanin pigments (Zou *et al*., 2024).

In this study, freshly harvested fruits (i.e. the "first reference" group, see Materials and Methods) displayed the usual pericarp protrusions, along with well-differentiated tissues in their exocarp (i.e. the cuticle, epidermis, and sclerenchymatous tissue with 2–3 layers of brachysclereids in Figure 4A). Additionally, the sclerenchymatous tissue was elastic and contained many cells with lignified walls that imparted mechanical resistance to the fruit (Figure 4A and B). The mesocarp, however, consisted mostly of anthocyanin-containing parenchymal cells (Figure 4B and C). Several vascular bundles were also found distributed along the spongy parenchyma (a part of the mesocarp) and are observed in greater detail in Figure 4A and D, along with endocarp cells (the small, non-lignified cells with thin walls). Even though micro-cracks (Figure 5A, B, D, E, G, H, J, K) were present in all fruit – including the freshly harvested reference group – those stored inside Xtend® bags had smaller and shallower pericarp fissures (Figure 5J and K). As a result, both WL and color loss were noticeably reduced in this group.

In litchi, the mesocarp interior becomes spongy prior to aril expansion and maturity. This both facilitates its separation from the endocarp and allows the pericarp to keep pace with aril growth, preventing fissures from forming (Pan and Xie, 1996; Wang *et al*., 2000; Huang *et al*., 2004). The spongy parenchyma also facilitates gaseous exchange with the fruit interior (Hieke, 2000). However, as the cytoplasm in its cells steadily contracts during storage, the walls will often distort and break, causing certain areas of the mesocarp to collapse. This effect was certainly observed in all treatments, independently of MAP (Figure 5A, D, G, and J); nevertheless, no histological changes occurred in the aril of most fruit stored at 3°C (including those hydrocooled and stored at 3 ºC inside Xtend® bags; see Figure 5I and L). In fact, the size of the aril cells in all treatments (60.3–79.8 μ long x 41.4–59.8 μ wide) did not vary

significantly as indicated by SEM imaging (Figure 5C, F, I, and L). Such results agree with prior observations by Underhill and Simons (1993), who report aril quality to be unaffected by pericarp dehydration and browning.

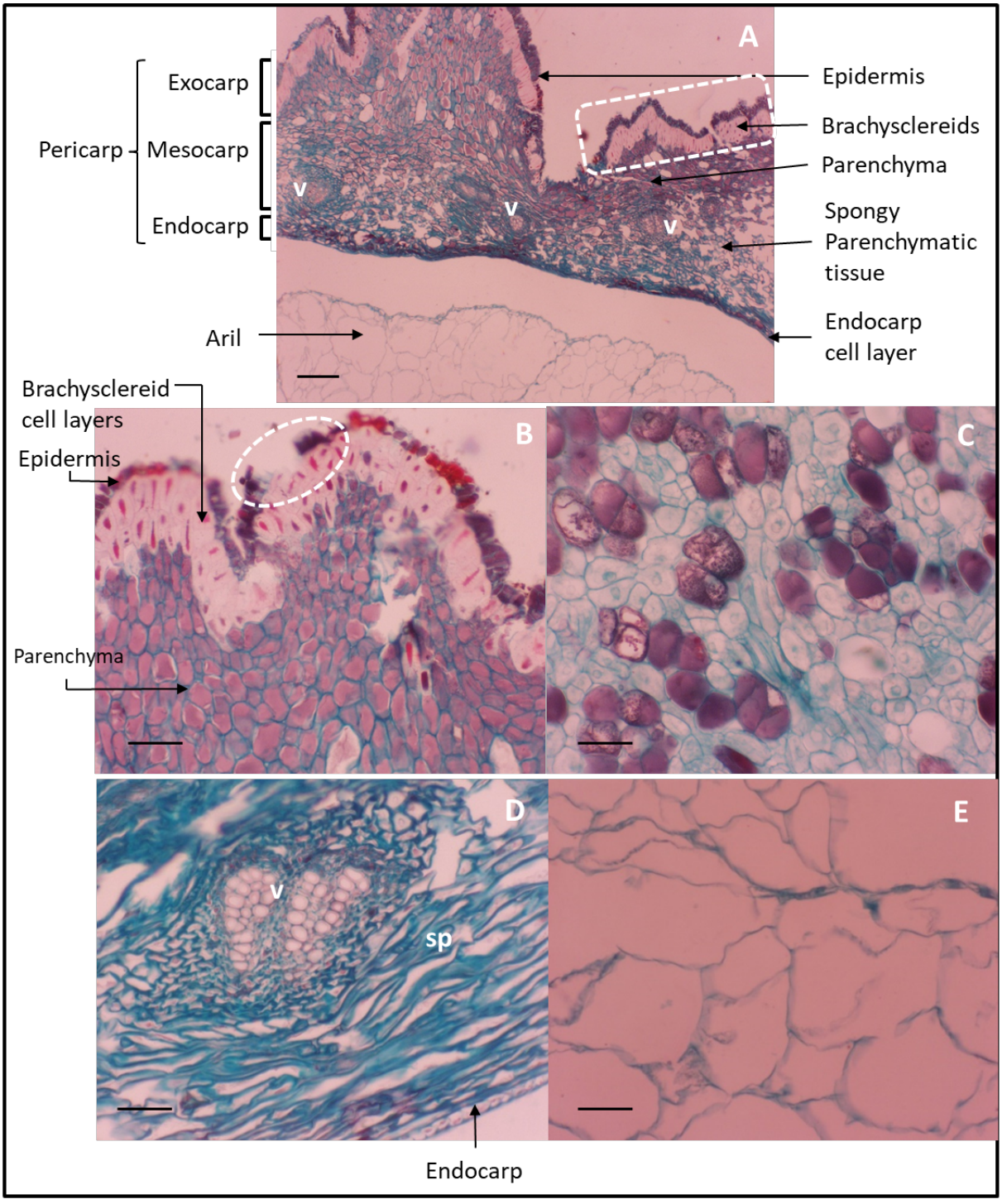

Figure 4. Optical microscopy cross-sections of freshly harvested litchi fruit. A: pericarp and aril tissues; B: detailed view of the exocarp (epidermis and highly-lignified brachysclereid cells) and mesocarp (parenchymatic cells); C: outer mesocarp cells with anthocyanin-filled vacuoles (i.e. pigmented cells in image); D: detailed view of the mesocarp (vascular bundles in spongy parenchyma) and endocarp; E, aril cells. Dotted rectangle shows characteristic protuberances in the litchi pericarp; dotted oval a micro-crack in the epidermis. sp: spongy parenchyma, v: vascular bundles. Bar = 287.5 μm in A and 112.5 μm in B–E.

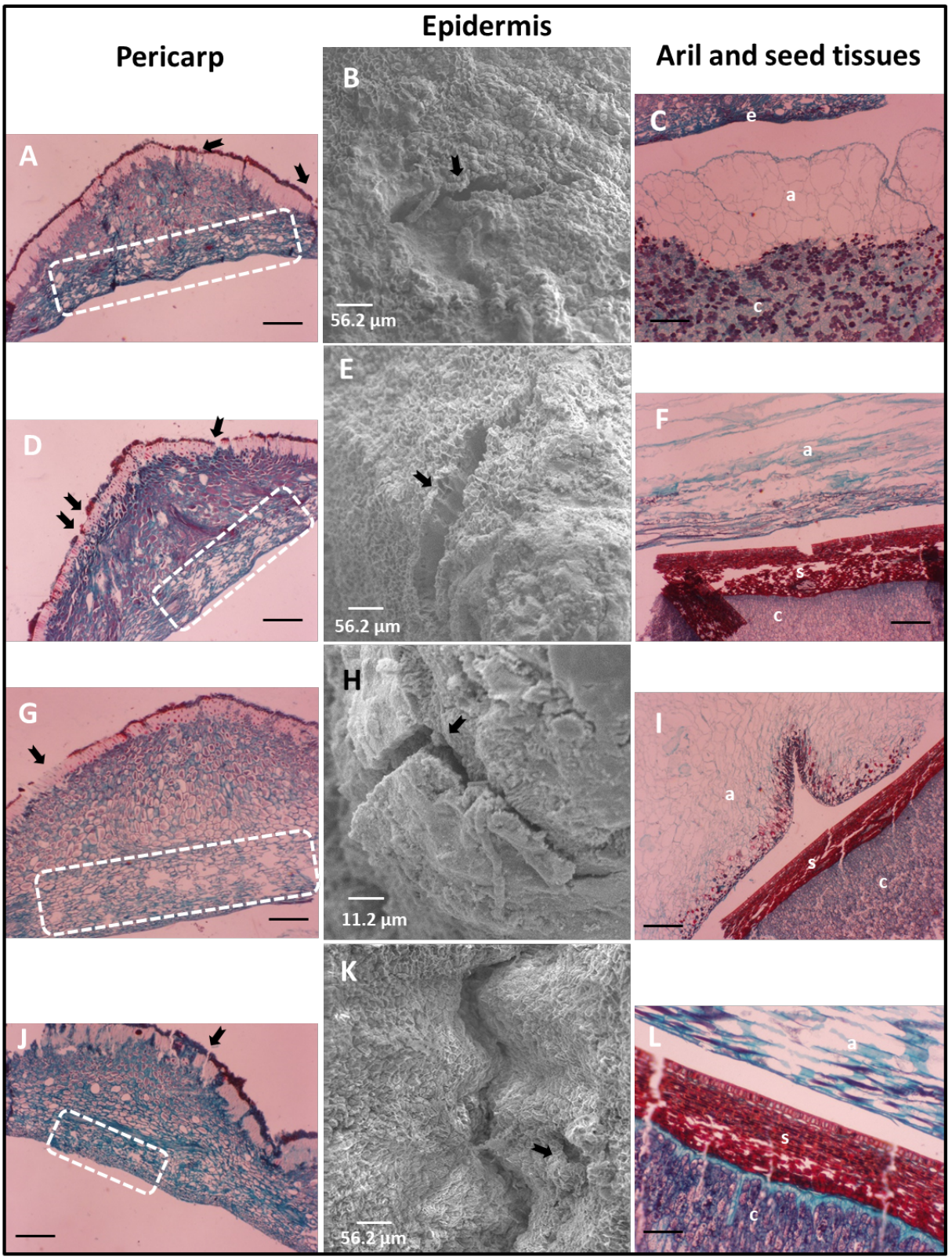

Figure 5. Histological changes in litchi fruit as a function of pre-storage and storage conditions: optical and scanning electron microscopy (SEM) images. A, B, and C: freshly harvested, ripe fruit; D, E, and F: stored at 25°C for 6 d; G, H, and I: stored at 3°C for 10 d; J, K, and L: hydrocooled and MAP-stored at 3°C for 10 d. Dotted rectangles show broken and/or distorted cells in the spongy parenchyma; arrows micro-cracks in the epidermis. a: aril, e: endocarp, c: cotyledon tissue, s: seminal cover. Bar = 287.5 μm in A, C, D, F, G, I, J, and L; 56.2 μm in B, E, and K; and 11.2 μm in H.

Past studies have emphasized the need for fast and efficient cooling of horticultural products prior to refrigerated transport or storage, and this has generally resulted in better quality products with longer postharvest lives. For instance, pre-cooling litchi with iced-water (0–2 ºC) for 2–3 h is usually more effective than cooling with forced-air (Jiang *et al*., 2003). Similarly, rapid cooling inside microperforated plastic bags (e.g. down to 4 ºC in 15 min) successfully preserves litchi color and taste during refrigerated storage at 5 ºC, and fruits cooled this way can have shelf lives of 2–4 days if adequately maintained at 20 ºC (Farina *et al*., 2017). Nevertheless, our results indicate that fast cooling is not always beneficial to fruit quality (e.g. forced-air cooling), and can even be detrimental in certain cases (e.g. hydrocooling). The reason for this may lie in the susceptibility to CI of cv. Brewster and varieties just like it.

Litchi cv. Brewster is, after all, a tropical crop that is far more susceptible to CI than fruits of temperate origin, which is the reason why higher temperatures (e.g. 7 ºC) are generally used for its refrigerated transport and storage. This susceptibility is also reflected in the temperature range that can ultimately be tolerated and used for cooling. Thus, media close to 0 ºC (whether air or water) should only be applied to 1) temperate crops, or 2) tropical and subtropical species resistant to early symptoms of CI. For all other crops and/or fruit varieties, cooling temperatures higher than 1.0 ºC should be used instead. The implications of these findings are especially relevant for Mexico given the significance of litchi as a perishable commodity. Similar studies should also be conducted on other tropical and subtropical species (and varieties) that are actively produced and commercialized in this country.

Conclusion

Operating data indicated that both equipment prototypes used to cool *Litchi sinensis* Sonn. cv. Brewster functioned adequately; however, there were no beneficial effects to forced-air cooling or hydrocooling under the storage conditions used (7 ± 1 ºC, 95 % RH, 28 days) except for a decrease in weight loss among forced-air treated fruit. Histological analysis confirmed the presence of chilling injury in the pericarp of hydrocooled litchi, and storage at 3 ºC preserved color and delayed browning but had no effect on either generalized drying or the formation of microfractures on the fruit epicarp. Modified atmosphere packaging, however, reduced the size and depth of these fissures and helped preserve color for 10 days.

Collectively, these results question the traditional method of applying cooling to tropical fruits – especially to chilling injury-sensitive produce like cv. Brewster – where hydrocooling is not generally recommended due to the rapid decline in temperature. In such cases, only air cooling should be applied – with an operating temperature clearly set above 1.0 ºC (i.e. above the temperature used in this study, for example: 3–5 ºC). Storage should then proceed at 7 ºC, preferably inside polymeric bags made of low water vapor transmission rate-polyolefins so that modified atmospheres are generated. The latter is especially true if fruits are to be transported or stored for extended periods, as modified atmospheres significantly reduce the early symptoms of chilling injury. Future research should contrast the effects of forced-air cooling with room cooling, as well as examine the inclusion of a conditioning treatment at 20 ºC before actual cooling and refrigeration.

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