Emirates Journal of Food and Agriculture. 2020. 32(5): 376-383
doi: 10.9755/ejfa.2020.v32.i5.2108
http://www.ejfa.me/

RESEARCH ARTICLE

Influence of storage period and shelf-life on the incidence of chilling injury and microbial load in "Angeleno" and "Larry Ann" plums

J. Rocha-Pimienta[1], J. Llera-Oyola[1], M.E. Bote[1], M.C. Ayuso-Yuste[2], M.J. Bernalte[2], B. Velardo[1], Jonathan Delgado-Adamez[1]*

[1]Technological Institute of Food and Agriculture (INTAEX). Avda. Adolfo Suárez s/n, 06007 Badajoz, Spain, [2]Agriculture Engineering School, University of Extremadura. Avda. Adolfo Suárez s/n, 06007 Badajoz, Spain

ABSTRACT

Refrigeration is the most widely used post-harvest technology for increasing commercial life (shelf-life) of plums after harvest. Nevertheless, low-temperature conservation is limited by chilling injury (CI), leading to rejection by consumers. The objective of the present work was to characterize biotic and non-biotic chilling injures that lead to quality loss in plums during post-harvest storage. *Larry Ann* and *Angeleno* plums were stored at 1.0 ± 0.5 °C and 90.0 ± 2.0% RH for 4 and 8 weeks and after this period the plums were stored either 3 or 6 days at 20ºC for evaluating changes in their shelf-life. Pulp firmness, post-harvest losses, presence of chilling injuries and the count of mesophilic microorganisms, molds and yeasts were determined after harvest and after storage plus shelf-life. In general, a drastic decrease in the growth of bacteria, mold and yeast during post-harvest storage at 1°C was observed, following an increased microbial growth during shelf-life storage. Sensitivity to low temperature was cultivar-dependent. *Larry Ann* plums showed higher pulp firmness reduction and lower impact of CI than *Angeleno*, with an increased microbial spoilage. Plums that presented cold-damaged or physiologically compromised tissues suffered from faster decomposition and provided a better substrate for microbial growth than non-damaged plums.

Keywords: Chilling injury; Decay; Dehydration; Firmness; Food-quality; *Prunus salicina.*

INTRODUCTION

Food loss has become a global problem and, as such, it is comprised as the objective 12.3 of the 2030 Sustainable Development Goals proposed by the United Nations: to halve the amount of food wasted along production and supply chains, including post-harvest losses (UN, 2015). In this article we study plum production from post-harvest preservation to commercialization. Plums are stone fruits that present a very limited postharvest life, mainly due to softening and mechanical injuries, as well as diseases that cause market rejection (Amorim et al., 2008; Zhang et al., 2010). Fruit losses caused by decay are estimated to be 5-10% when postharvest fungicides are used; without fungicide treatment, losses may reach 50% or more (Karabulut et al., 2010).

Harvest losses in plums can be classified as postharvest and pre-harvest mechanical injuries, physiological disorders, pre-harvest and post-harvest diseases (Amorim et al., 2008). Plums produced in Extremadura, the region where the present study was carried out, are exported to foreign countries, and therefore for its commercialization there is a necessity for studying the preservation of the fruits in long storage periods, including the surface microbiota that can potentially spoil the plums.

Several post-harvest treatments have proven to be effective for maintaining the global quality in plums, for example 1-Methylcyclopropene (Manganaris et al., 2008; Minas et al., 2013; Valero et al., 2016; Velardo-Micharet et al., 2017; Lin et al., 2018) and modified atmospheres (Cantin, H. Crisosto, & R. Day, 2008; Nunes et al., 2019). Curing treatments has been also used to reduce the incidence of CI (Sun et al., 2010).

Refrigeration is the most widely accepted technique for preventing and reducing post-harvest losses, since low

***Corresponding author:**
Prof. Jonathan Delgado Adámez. Technological Institute of Food and Agriculture (INTAEX). Avda. Adolfo Suárez s/n, 06007 Badajoz (Spain). **Tlf:** (0034) 9240142670; **Fax:** (0034) 924012674. **E-mail:** jonathan.delgado@juntaex.es

Received: 10 March 2020; **Accepted:** 1 June 2020

temperature reduces enzymatic and microbial activity, respiratory rate, transpiration and delay ripening (Yahaya & Mardiyya, 2019). Nonetheless, sensitive varieties may develop chilling injury (CI) when exposed to low temperatures. CI develops faster and more intensely in stone fruits stored in the range of 2.2-7.6 ºC than fruit stored at 0 ºC or below and above their freezing point (Lurie & Crisosto, 2005). Flesh browning and translucency are two of the main CI symptoms observed in plums. Flesh browning (internal breakdown) appears as a brown discoloration of the flesh, and flesh translucency (gel breakdown) manifests itself as a translucent gelatinous breakdown of the mesocarp tissue around the stone (Candan et al., 2008; Manganaris et al., 2008; Taylor et al., 1993). Flesh bleeding occurs as an accumulation of red pigment either around the stone, or immediately beneath the epidermis (Manganaris et al., 2008).

Microbial contamination by several fungi affects quality and shelf-life of fruits, generating big losses during postharvest storage even when they are stored at refrigeration temperature (Hussain et al., 2015). Although contamination can occur during cultivation, it only proliferates and causes damages after harvest, when fruit defenses are reduced or eliminated (Tournas & Katsoudas, 2005). Unsuitable practice and conditions of transport and commercialization contribute to bacteria and fungi growth (Grzegorczyk et al., 2017). The main postharvest diseases of stone fruit are brown rot, caused by *Monilia fructicola*, also commonly known by their teleomorph name *Monilinia fructicola* (G. Winter) Honey; and *Rhizopus* rot, caused by *Rhizopus stolonifer* (Erhenb.:Fr.) Vuill. (Gonçalves et al., 2010).

Alternative control methods to synthetic fungicides have been developed, such as biological control (Zhang et al., 2010), hot water treatment (Karabulut et al., 2010) and coating with commercial waxes (Gonçalves et al., 2010). Despite their good results against stone fruit postharvest pathogens, these researches were conducted under experimental conditions, therefore it is necessary to study their effectiveness throughout the supply chain and marketing of stone fruit.

The objective of the present work is to characterize biotic and non-biotic chilling injuries that plums develop during refrigeration storage, to assess to a subsequent quality loss during post-harvest storage and to evaluate the correlation between damage caused by CI and molds and yeast charge.

MATERIALS AND METHODS

Plant material

Plums (*Prunus salicina* Lindl. cv. *Larry Ann* and *Angeleno*) were harvested from a local farm in Extremadura (Spain) at commercial ripening stage based on size, colour and firmness. For each cultivar, the plums were manually picked and transferred in less than 2 hours to the laboratory for their pre-cooling (temperature below 3ºC in the center of the fruit) and selection according size (60-66mm and 53-57mm in *Larry Ann* and *Angeleno* cultivars, respectively) of the plums that didn't present any defect. For the evaluation of the CI, we designated sets of 35 fruits per cultivar to undergo refrigerated storage. One set of each cultivar was stored at 1.0±0.5 °C and 90.0±2.0% HR for 4 and 8 weeks. Subsequently, said fruits were transferred to storage at 20 ºC for 4 days; afterwards, a shelf-life period was simulated at 20ºC for 3 days in *Larry Ann* and for 6 days in *Angeleno* cultivars. Another set of 30 fruits of each cultivar were stored for maturation at 20ºC for 5 days in *Larry Ann* and for 6 days in *Angeleno* plums.

Firmness

Firmness measurements were taken at two diametrically opposite points of the equatorial zone after removing a piece of skin of ~1 cm in fifteen fruits. It was determined using a Stable Micro Systems Texture Analyzer TA-XT2i (Aname, Pozuelo, Madrid, España) with an 8 mm diameter cylindrical probe. Force/deformation curves were determined and the maximum force (N) was calculated.

Postharvest losses

Fruit water loss and chilling injuries were considered as postharvest losses. A sample of 15 intact fruits per cultivar and sampling was analysed. Water loss was determined both numerically, through weigh loss occurred during postharvest storage, and visually, by the wrinkling of fruit shoulders. Weight loss was determined as the weight difference between pre- and post-refrigerated storage and expressed as percentage with respect to the basal value.

Chilling injuries were determined visually by cutting 15 fruits in half for the evaluation of the pulp. Even the minor incidence of CI was considered in the present study.

Counting techniques

Mesophilic microorganisms (ISO 4833-1, 2014) and mold and yeast (ISO 21527-1, 2008) were evaluated in 6 fruits randomly selected from an entire set and quadruplicated.

Isolation and determination of molds

The material from the affected area was aseptically transferred to Petri dishes with malt extract agar (5-7 days at 25ºC). The several filamentous fungi were isolated from this primary culture and transferred to malt extract agar and Rose Bengal agar Base. The identification of the genus and species was made by microscope observation with a taxonomic key (Pitt & Hocking, 2009; Samson et al., 2002), and the computerized system MicroStation™ BIOLOG.

Statistical analysis

Data was analyzed by Statistical Package SPSS 17.0 version for Windows (SPSS Inc., Chicago, lll., U.S.A.). Tukey's test for pairwise comparison was used to determine significant differences at a confidence level of 5%. Pearson correlation was carried out to understand any relation between analyzed parameters. Mean values with standard deviations are reported.

RESULTS AND DISCUSSION

Preliminary characterization

A routine characterization of the plums was carried out at harvest: *Larry Ann* presented 35.33±6.37 N of firmness, 15.4±2.04 °Brix of sugar content, and 1.46±0.2% of malic acid as an acidity measure. *Angeleno* plums presented 30.14±4.44 N of firmness, 21.5±0.26 ºBrix of sugar content and 0.82±0.02% of acidity at harvest.

Firmness

Larry Ann plums lost 9% of their initial firmness after 5 days of shelf life from harvest (Fig. 1). After 4 weeks of postharvest storage at 1.0 ºC a 53% reduction of firmness was observed, reaching a mean value of 16,73 N. Previous studies considered these values as "ready to buy" for a variety of plum cultivars (Valero et al., 2007). After 8 weeks of storage at 1.0ºC, it was observed an increase in firmness of fruits. probably due to a CI called leatheriness. This disorder is characterized by a change in the texture of the pulp tissue on the ripe fruit from slippery and juicy to a firm and dry pulp tissue caused by cell wall synthesis that thickens the tissue underlying the skin (Candan et al., 2006). *Angeleno* maintained the initial firmness after the first 6 days of shelf-life from harvest (Fig. 1). During postharvest storage, fruits reduced their firmness by 16 and 20% at 4 and 8 weeks of storage, respectively. *Angeleno* plums presented values of firmness over 20 N, values comprised in the "ready to buy" margin during the whole experimental period. For this reason, contrary to *Larry Ann*, *Angeleno* plums show an excellent aptitude for postharvest storage (Candan et al., 2011; Velardo et al., 2010). These differences might be due to the different maturation process in both cultivars. *Larry Ann* plums show a typical climacteric ripening behaviour and their quality properties change sharply from the moment of harvest (Sadka et al., 2019). However, *Angeleno* is a suppressed climacteric cultivar with a slower maturation and, therefore, longer post-harvest life (Fernandez i Marti et al., 2018).

Chilling injuries

The model proposed by Lyons (1973) for the underlying mechanism of CI is generally accepted. CI is mainly related to changes in membrane permeability associated with a transition of the membrane lipids from a more flexible liquid-crystalline solution to a solid gel-like structure (Lyons, 1973; Candan et al., 2008; Zhou et al., 2001). This first event leads to a cascade of secondary ones, which result in a loss of the mandatory control and an unbalanced metabolism, cellular autolysis, and finally, the development of a wide variety of chilling symptoms (Wang, 1989).

In general, *Larry Ann* plums showed higher sensitivity to CI compared to *Angeleno* (Figs. 2 and 3). At the fourth week of storage, water loss appeared in both varieties of plums. Pulp pigmentation presented differences according to the

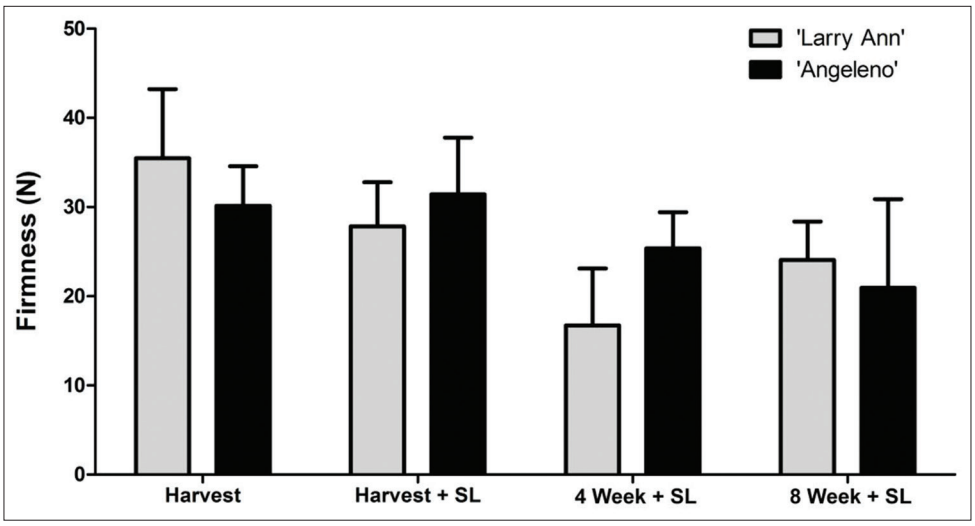

Fig 1. Firmness evolution of Larry Ann and Angeleno plums during cold storage (at 1 °C) and shelf-life (at 20 °C). The shelf-life periods for Larry Ann were 3 or 5 days depending on the set (refrigerated or not refrigerated) and of 6 days for Angeleno. Comparison between the two cultivars studied for each period tested presented no significant differences (p>0,05). Results are shown as mean ± standard deviations of the firmness at harvest (Harvest), at harvest after shelf life (Harvest + SL) and after 4/8 weeks of cold storage plus a period of shelf-life (4 Week + SL and 8 Week + SL).

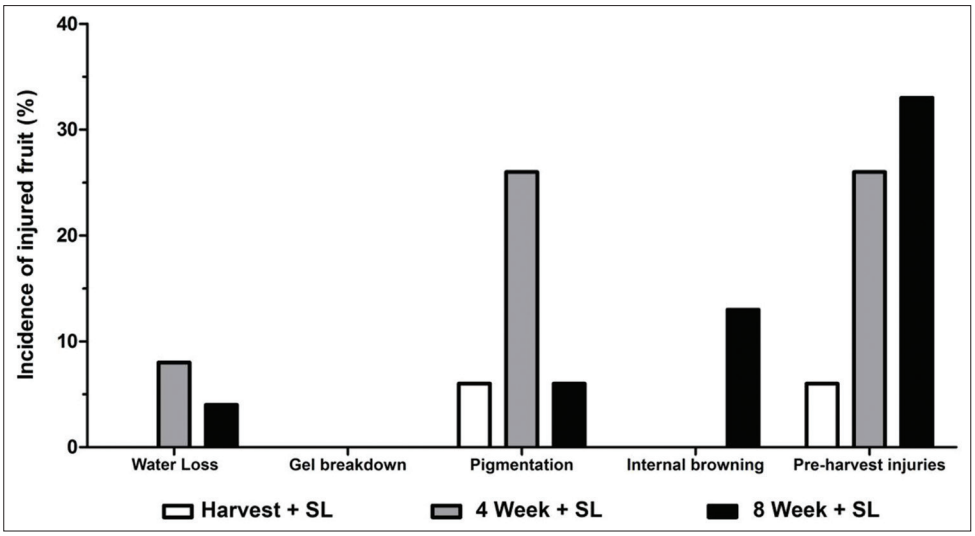

Fig 2. Effect of cold storage and shelf-life in water loss, gel breakdown, pigmentation, internal browning, and pre-harvest damage in Angeleno plums. Results are shown as the percentage of fruits that displayed any of the injuries studied at harvest plus a period of shelf-life (20 ºC) of 6 days (Harvest + SL) or after 4/8 weeks of cold storage plus shelf-life (1 °C) (4 Week + SL and 8 Week + SL).

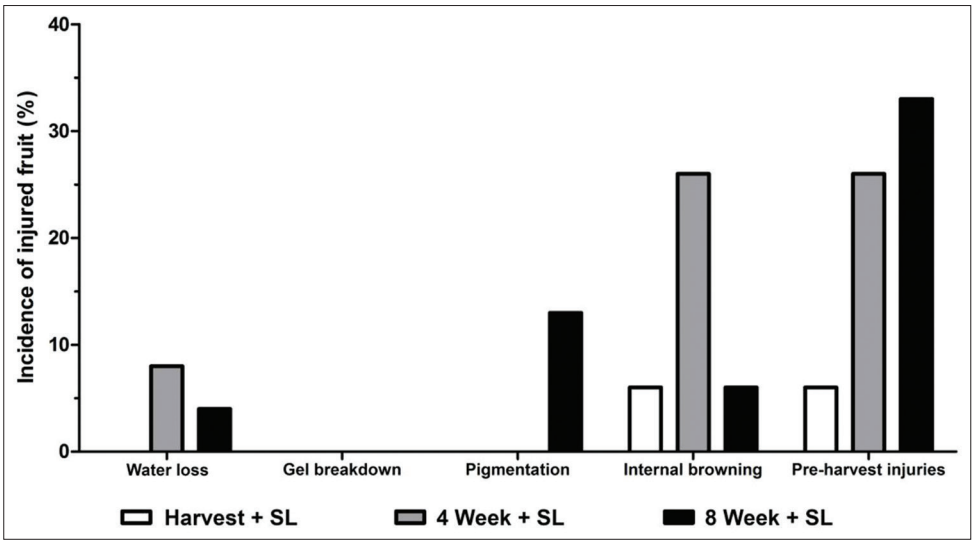

Fig 3. Effect of cold storage and shelf-life in water loss, gel breakdown, pigmentation, internal browning, and pre-harvest damage in Larry Ann plums. Results are shown as the percentage of fruits that displayed any of the injuries studied at harvest plus a period of shelf-life (20 ºC) of 3 days (Harvest + SL) or after 4/8 weeks of cold storage plus shelf-life (1 °C) (4 Week + SL and 8 Week + SL).

Gel breakdown didn't appear in the stored fruit. This CI is dependent of the refrigeration time and it is not observed in this study. Gel breakdown had been previously described in *Larry Ann* plums with higher incidence than in non-climacteric cultivars. *Angeleno* plums can behave as non-climacteric due to its suppressed climacteric behaviour (Candan et al., 2008). *Angeleno* presented pre-harvest damages that were not found in *Larry Ann*.

Microorganism count

The superficial microbiota of plums was evaluated counting mesophilic microorganisms as a quantitative indicator of the superficial charge of fruits, as well as the counting of fungi and molds because of their importance in the post-harvest rotting. Thus, it has been observed that storage at 1 ºC, both during 4 and 8 weeks, drastically reduced the microbial charge and prolonged shelf-life (Figs. 4 and 5). In both cultivars, an increase in molds was observed at 8 weeks of storage (Fig. 5), which is mainly due to *Alternaria* and *Cladosporium* presence (Table 1), as these molds can grow at low temperatures (Tournas, 2005). The plums that presented chilling injuries were highly susceptible to microorganism impairment. Therefore, the correlation between damage and molds and yeast charge (Table 2) was evaluated. Broadly speaking, a correlation between all the parameters studied was found, except for browning and flesh pigmentation in *Angeleno* and gelation in *Larry Ann*. Gelation is an internal damage that does

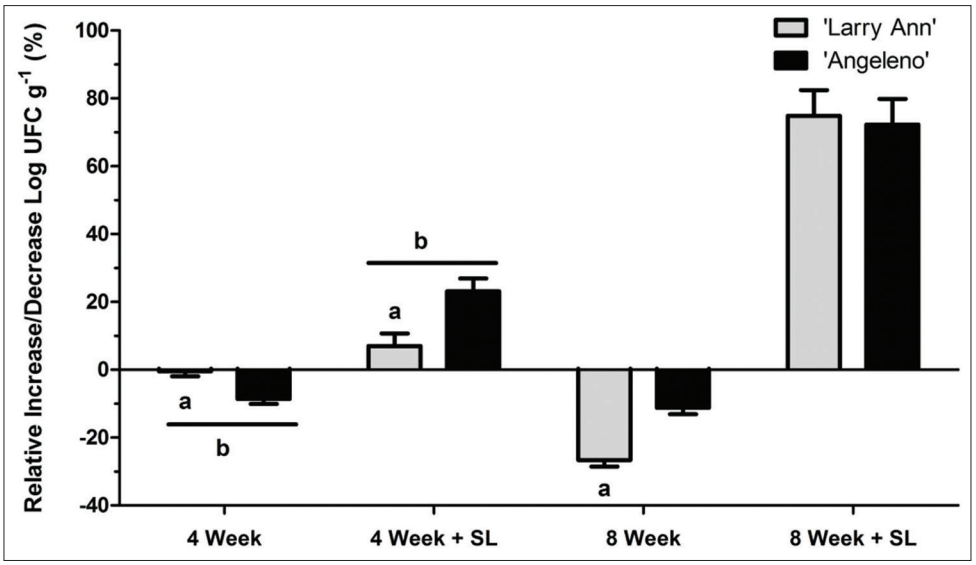

Fig 4. Effect of storage and shelf-life in mesophilic aerobic bacteria charge in Larry Ann and Angeleno plums. Results are expressed as the mean ± standard deviations of the relative variation of the logarithm of CFU (Colony Forming Units) of total mesophilic aerobic bacteria detected in both cultivars of plums: after 4 weeks of cold storage (1 °C) with a period of shelf-life of 3 days for Larry Ann and 6 days for Angeleno (20 °C) (4 Week + SL) and without it (4 Week); and after 8 weeks of cold storage with a period of shelf-life of 3 days for Larry Ann and 6 days for Angeleno (8 Week + SL) and without it (8 Week). a: $p<0.05$ *vs* 8 weeks + SL; b: $p<0.05$ between cultivars.

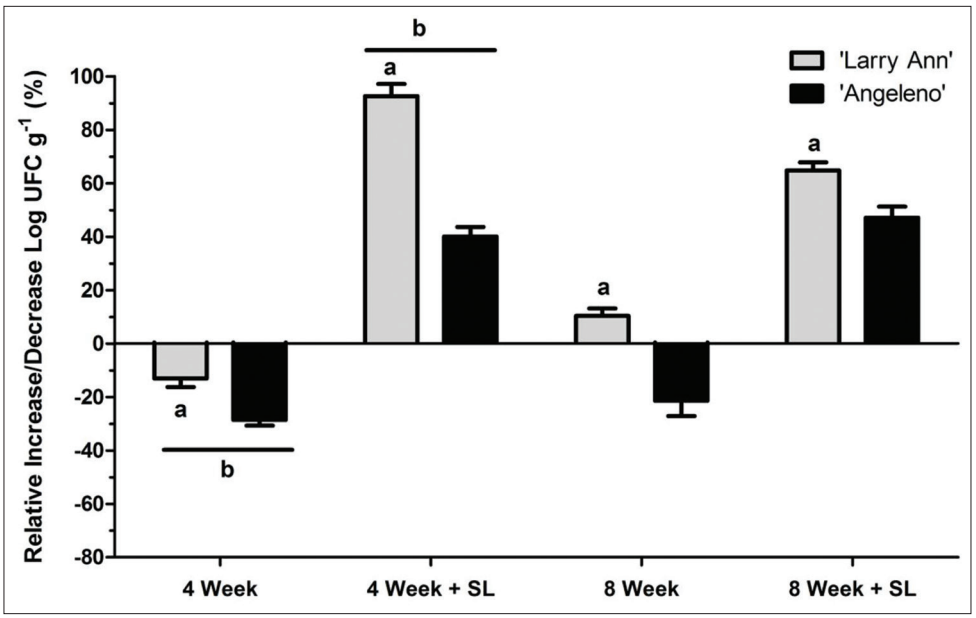

Fig 5. Effect of storage and shelf-life in mold and yeast charge in Angeleno and Larry Ann plums. Results are expressed as the mean ± standard deviations of the relative variation of the logarithm of CFU (Colony Forming Units) of total molds and yeasts detected in both cultivars of plums: after 4 weeks of cold storage (1 °C) with a shelf-life period of 3 days for Larry Ann and 6 days for Angeleno (20 ºC) (4 Week + SL) and without it (4 Week); and after 8 weeks of cold storage with a shelf-life period of 3 days for Larry Ann and 6 days for Angeleno (8 Week + SL) and without it (8 Week). a: $p<0.05$ *vs* 8 weeks + SL; b: $p<0.05$ between cultivars.

not affect the epidermis and, therefore, do not contribute to the impairment of the superficial defenses against opportunistic microorganisms. This data supports the hypothesis that the impairment of fruits caused by CI facilitates the growth of opportunistic microorganisms. Fig. 6 consist of photographs depicting the damages detected in the studied plums that were a consequence of the handling and preservation processes studied in this work, at harvest, in the post-harvest cold storage and at the end of the shelf-life period.

Fungi isolation and identification

Filamentous fungi that caused pathology in harvest, storage and shelf-life were identified in both varieties (Table 1). Pathologies associated to fungi in Angeleno, either in

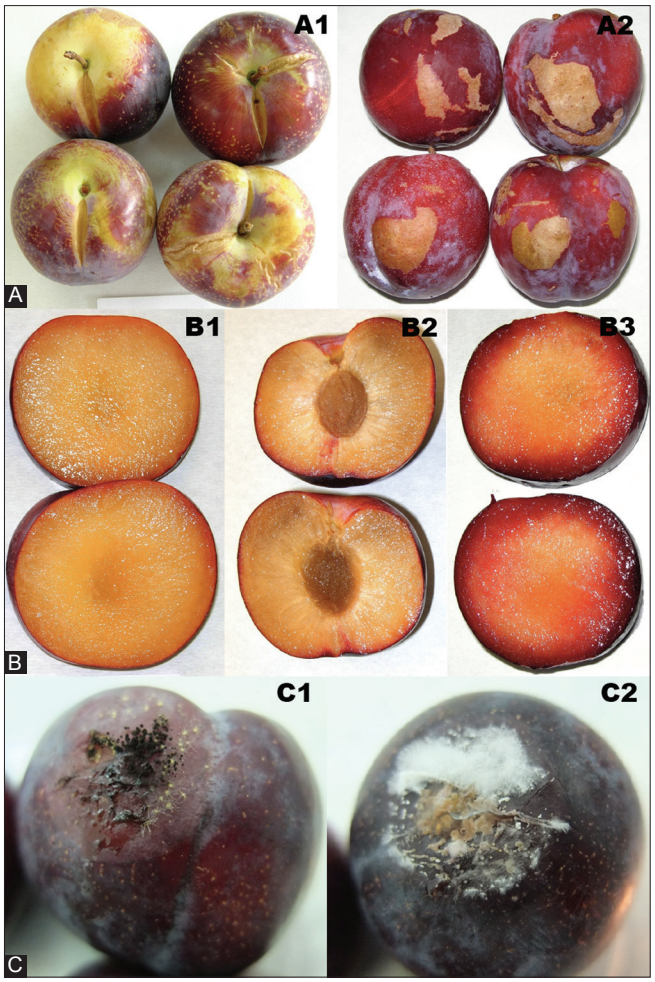

Fig 6. Images of Larry Ann and Angeleno plums at different times in the experimental design. A: show plums at harvest with superficial mechanical damages caused during harvest, previous to cold storage. A1 shows cracks caused by tree branches in Larry Ann and A2 shows damages caused in Angeleno plums due to bruising against the tree leafs. B: CI caused at the end of cold storage in Larry Ann plums. B1 is an image of a control group plum that has not been cold storaged; in B2 pulp browning is developed and B3 shows pulp bleeding. C: Development of molds on the surface of plums after shelf-life period (20 °C, 3 days for Larry Ann and 6 days for Angeleno).

Table 1: Mold identification in "Angeleno" and "Larry Ann" plums, at harvest, storage and shelf-life

	Angeleno	Larry Ann
Harvest	*Alternaria alternata* *Alternaria infectoria* *Aspergillus niger* *Aspergillus sydowii* *Fusarium oxysporum* *Penicillium chrysogenum* *Penicillium ssp* *Mucor hiemalis* *Rhizopus stolonifer*	*Alternaria alternata* *Aspergillus sydowii* *Penicillium citrinum* *Rhizopus stolonifer* *Cladosporium cladosporioides* *Ulocladium chartarum*
Storage	*Alternaria alternate* *Cladosporium cladosporioides* *Cladosporium spp* *Penicillium citrinum* *Penicillium expansum*	n.d.
Shelf-life	*Alternaria alternata* *Cladosporium cladosporioides* *Ulocladium chartarum* *Acremonium strictum* *Penicillium chrysogenum* *Penicillium expansum* *Penicillium citrinum*	n.d.

Table 2 :Study of the correlation between damage and molds and yeast charge in "Angeleno" and "Larry Ann" plums

	Angeleno		Larry Ann	
	R^2	*p*	R^2	*p*
MY *vs.* Dehydration	0.949	0.0010	0.736	0.0288
MY *vs.* Internal browning	0.050	0.6685	0.380	0.1027
MY *vs.* Pigmentation	0.535	0.0985	0.860	0.0077
MY *vs.* Firmness	0.996	<0.0001	0.879	0.0057
MY *vs.* Pre-harvest damage	0.7362	0.0288	-	-
MY *vs.* Gel breakdown	-	-	0.568	0.0834
MY *vs.* Rots	-	-	0.7716	0.0213

storage or shelf-life, were not detected. However, in Larry Ann rot was detected in the three stages of the study. It is important to highlight the high presence of *Cladosporium* and *Alternaria* genus in the plums. *Cladosporium* is responsible for cladosporiosis, one of the most common diseases in plums (Amorim et al., 2008). *Cladosporium* presence was detected in both cultivars studied. On the other hand, symptoms of cladosporiosis are minor because the growth of this mold is limited to small areas surrounding the place where the pathogen came into the fruit (Mari et al., 2019). *Alternaria spp.* can cause deterioration in stone fruits (Moosa et al., 2019) although, in general, with minor economic repercussion. *Rhizopus stolonifer*, suggested as one of the main causes of rotting losses in plums (Mari et al., 2019), was also detected. Blue mold (*Penicillium sp.* and *Penicillium expansum*) (Louw & Korsten, 2016), rotting by mucor (*Mucor piriforme*) and bitter rot (*Colletotrichum gloeosporioides* and *C. acutatum*) (Børve & Vangdal, 2007) were present in the plums.

There are other microorganism responsible for deterioration of stone fruits (such as *Monilia fruticola* and *Botrytis cinerea*) (Børve & Vangdal, 2007; Snowdon, 1990) that didn't proliferate in the samples.

Rot losses

Angeleno plums did not rot in the periods of shelf-life set after harvest or after cold storage, whereas Larry Ann plums rot increasingly as cold storage times where longer, reaching values close to 12% at the end of the study (Fig. 7). That could be due to a higher sensitivity to microorganisms of this variety, caused by higher prone to CI appearance with respect to *Angeleno*. CI in plums are associated to abnormalities in the cellular wall metabolism (Manganaris et al., 2008) and to changes in the permeability of the membrane (Candan et al., 2008) leading to a natural barrier's

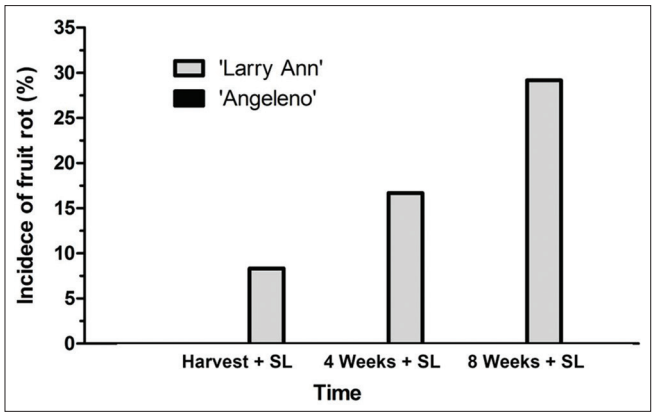

Fig 7. Effect of storage and shelf-life in the incidence of fruit rot in Larry Ann and Angeleno plums. Results show percentage of rot incidence after a shelf-life period of 3 days for Larry Ann and 6 days for Angeleno at 20 ºC for the plums at harvest (Harvest + SL), after 4 weeks of cold storage (1 ºC) plus shelf-life (4 Weeks + SL) and after 8 weeks of cold storage plus shelf-life (8 weeks + SL).

weakening against microorganism infection. *Angeleno* plums showed more resistance to CI than *Larry Ann* plums, resisting longer cold storage times with a better microbial quality during the shelf-life period they were subjected to afterwards. Therefore, it seems that plums from *Angeleno* cultivar would better resist exportation periods while in cold storage without suffering microbial quality loss such as the one displayed by *Larry Ann* plums.

CONCLUSIONS

In general terms, we found correlation between the abiotic parameters studied and the mold and yeast charge in both plum cultivars, being the *Angeleno* plums more resistant to CI and microbial colonisation, given its suppressed-climacteric maturation, while *Larry Ann* plums were the most susceptible of deterioration, due to its climacteric maturation. The longest period of cold storage caused CI that were responsible for a faster decrease of microbial quality of the plums along their shelf-life. While cooling is essential to maintain the quality and extend shelf-life of fruits, there are physiological parameters of the fruit crucial to the viability of its commercialization such as its maturation mechanism. In the case of plums, long cold storage periods must be followed by a fast commercialization due to a reduction of their shelf-life.

Acknowledgements

The present work belongs to the project "Red de Investigación Transfonteriza de Extremadura, Centro y Alentejo (RITECA), founded by FEDER through the "Programa Operativo de Cooperación Transfronteriza España-Portugal" (POCTP) 2007-2013 and Junta de Extremadura.

Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contributions

JRP conceptualized the study, wrote the original draft and conducted the experiments. JLO conducted the experiments and carried out formal analysis of the data. MEB conducted the experiments and curated data. MCAY designed the methodology and statistical analyses. MJB supervised the experiments and performed data curation. BV provided visualization and resources. JDA supervised the study, designed the experiments and reviewed and edited the article to be published.

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