Investigation note

# Quality and post-harvest life of Mexican lemon fruits from trees with symptoms of HLB and asymptomatic

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## Abstract

In Mexico, the presence of Huanglongbing (HLB) in the producing areas of Mexican lemon, has been manifested in a reduction in production that, in the case of the Colima production area, has been estimated at 66.8% in the last 10 years. The effect of the disease on the quality of the fruits has been little studied, the reduction in size being reported. The objective was to evaluate the organoleptic and nutritive quality, related to the postharvest behavior of Mexican lemon fruits from trees with HLB and asymptomatic. Fruits were harvested from trees with and without symptoms of HLB from the INIFAP Fruit Experimental Field, Tecoman Colima, Mexico. Four treatments were established considering the origin of the fruits (trees with symptoms of HLB and asymptomatic) and two temperatures (environment: 22 ±2 ºC per 4 and 8 days, refrigeration: 9 ±1 ºC per 2 weeks plus 4 and 8 days to the environment). After each period, the following variables were evaluated: SST, titratable acidity, technological index, ascorbic acid (vitamin C), weight loss, color index and damages by cold. Regarding these variables, no significant differences were observed, at the time of harvest, between fruits of trees with symptoms of HLB and asymptomatic. The post-harvest behavior was similar during the two storage conditions and no differences in the variables were observed; however, the losses of weight, ascorbic acid and green color were greater in fruits from trees with HLB, in addition to having a higher incidence of cold damage. It is concluded that the quality, evaluated by the mentioned variables, of the fruits from trees with symptoms of HLB and asymptomatic is not significantly affected, except that the fruits harvested from trees with HLB are more sensitive to cold damage.

**Keywords:** *Diaphorina citri*, Huanglonbing, Mexican lemon, postharvest.

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Because of its varied use, the Mexican lemon (*Citrus aurantifolia* Swingle) is one of the fruit trees of commercial importance in Mexico; however, it is currently facing a severe production problem due to the presence of Huanglongbing (HLB), a devastating impact disease for citrus fruits (Manjunath *et al*., 2008) caused by the proteobacterium *Candidatus liberibacter* and transmitted by the psyllid asian *Diaphorina citri* Kuwayama (Boveé, 2006). According to Gottwald and Irey (2008) the presence of HLB in citrus fruits is responsible for losses of up to 65% of the yield, in Mexico it has been detected in various sweet citrus and acidic limes producing areas. In the case of Mexican lemons, it has become endemic in states such as Colima, where production has experienced a significant decrease, which in 2005 stood at 525 803 t and for 2014 at 174 615 t, signifying a decrease of 66.8% (SIAP, SAGARPA, 2016); In this regard, Robles *et al*. (2014) have reported that, in Mexican lemon trees with 75-100% of symptoms of the disease, their production is reduced up to 50%, with respect to asymptomatic, due to a significant reduction in flowering and fruiting capacity, to cause by the decrease of the photosynthetic activity of the damaged leaves (Robles *et al*., 2013).

It has been pointed out Bove (2006) that in fruits the symptoms are not specific; however, in trees with advanced symptoms of HLB the fruits produced are small, asymmetric and of low commercial quality, in addition to reducing the (%) juice and SST content. According to Robles *et al*. (2014), in the case of Mexican lemon, the main effect on quality is the reduction in size, not affecting the thickness of the peel, number of seeds developed and aborted (%) of juice, titratable acidity and SST; however, given the sensitivity of the fruits to weight loss, decrease in green color and the appearance of physiological disorders, the postharvest behavior of fruits from trees affected by HLB is unknown, since their demand in the national market is not it has been demerited. Therefore, the objectives of this research were to evaluate the effect on the organoleptic and nutritive quality, related to the post-harvest life of Mexican lemon fruits harvested from trees with and without HLB symptoms.

The experimental phase consisted in the harvest of a total of 600 Mexican lemon fruits of the Colimex variety of trees developed in the experimental field of the National Institute of Agricultural and Livestock Forestry Research (INIFAP) of Tecomán, Colima, Mexico, located at 40 masl, climate is warm, subhumid with rain in summer and semi-dry very warm; the average temperature is 25 ºC and the average annual total rainfall varies from 798.7 to 940 mm; of the total 300 fruits were cut from trees with symptoms of HLB (% of juice 39.1 ±3.4) and another 300 of asymptomatic trees (AS) (% juice 37.2 ±2.4).

Each batch of 300 fruits was subdivided into two of 150, one of which was stored at room temperature (20 ± 2 °C and 55 ±5% HR) and another at refrigeration conditions (9 ±1 ° C, 85 ±5% HR), with four treatments: $HLB_{amb}$, $AS_{amb}$, $HLB_{ref}$ and $AS_{ref}$, the fruits were stored for 4 and 8 days and those exposed to refrigeration for 2 weeks plus 4 and 8 days at room temperature, the latter to simulate conditions of marketing for consumers.

During the experiment, organoleptic quality variables (total soluble solids, titratable acidity and technological index in juice), nutritional quality (ascorbic acid content) and post-harvest life (external color, weight loss and cold damage index) were evaluated. The total soluble solids were measured applying the AOAC (2000) method, using a digital ATAGO PR-100 refractometer with 0-32% scale, the data were expressed as% SST, for the titratable acidity (AT) the methodology was applied described by the AOAC (2000), using for neutralization 0.1N NaOH and phenoftalein indicator in 2.5% alcohol solution, this variable was reported based on the content (%) of citric acid, with the equation% SST∗% juice/100, the technological index (IT) was calculated (Obeed and Harhash, 2006).

The content of ascorbic acid (vitamin C) in juice was determined by the method of Tillman (1990), performing the extraction with oxalic acid (0.5%) and the titration with 2,6-dichlorophenol-indophenol (0.02%), for the calculation of the content of this vitamin a standard curve was obtained and the data were expressed in mg 100 $mL^{-1}$. External color measured in the equatorial zone of the whole fruit by means of a Hunter Lab reflection colorimeter (Reston, Virginia, USA, model D-25) with CIELab scale calculating the color index (IC) with the color parameters L, a, b obtained ) for citrus fruits (1000a/bL) proposed by Jiménez *et al*. (1981), weight losses obtained by the difference in weight between the one obtained at the beginning of the storage and the one registered during the advance of the same, expressing the data as% PP, cold damage index (%) considering the number of fruits with symptoms of chopped and spotted epicarp (Wang, 2010).

For the statistical analysis of the data, a fruit was considered as an experimental unit, with a number of repetitions of: 10 (weight loss and color index) and 50 (cold damage, considering the analysis of organoleptic and nutritional quality as experimental unit five fruits and five repetitions. The determinations were made at 4 and 8 days in the ones stored directly at room temperature, as well as at the start and exit of refrigeration plus 4 and 8 days after transferring to the environment. The data were analyzed applying a generalized randomized block experimental design and means comparison tests by Tukey ($p \leq 0.05$), applying the SAS System V9 program (SAS Institue 2002).

**Storage at room temperature**

At the time of harvest, no significant differences were observed between fruits from trees with and without symptoms of HLB, in all the variables evaluated (Table 1), which coincides with that reported by Robles *et al*. (2013) who did not find significant differences in the content of juice, Brix and ascorbic acid; however, it is contrary to that reported by Bassanezi *et al*. (2009) in sweet oranges from Brazil where fruits harvested from trees with HLB had lower juice content and SST, as well as higher acidity. The weight losses increased significantly as the storage time advanced in both the fruits from trees with HLB and without HLB, but there were no differences between the two groups; similar response was observed with the color index, which decreased significantly as the storage time advanced, without differences between the two groups of fruits (Table 1), no greening problems were observed (greening) at the stylar end as in the case of oranges (Bassanezi *et al*., 2009).

**Table 1. Evaluation of some of the quality components of Mexican lemon fruits stored at room temperature (20 ±2 ºC) for 4 and 8 days.**

| Storage (days) | PP (%) | AT (% acid citric) | SST (%) | Index technological | IC (1 000a/bL) | Acid ascorbic (mg 100 $mL^{-1}$) |
|---|---|---|---|---|---|---|
| C/HLB | | | | | | |
| Harvest | 0 c | 7.7 a | 9.2 a | 2.6 ab | -13.2 a | 39.1 a |
| 4 | 6.5 b | 7.7 a | 9.2 a | 1.9 b | -11.8 b | 33.7 b |
| 8 | 12.4 a | 8.3 a | 9.9 a | 1.8 b | -8.7 c | 25.4 c |
| S/HLB | | | | | | |
| Harvest | 0 c | 7.7 a | 9.2 a | 3.3 a | -13.3 a | 43.6 a |
| 4 | 6.3 b | 7.7 a | 9.2 a | 2.3 ab | -11.2 b | 35.3 b |
| 8 | 11.9 a | 7.5 a | 8.9 a | 2.1 ab | -9.2 c | 28.2 c |

Means with the same letter in each column are not significantly different according to Tukey test ($p \leq 0.05$).

As for vitamin C, in both groups of fruits the concentration tended to decrease as storage progressed, as a result of the oxidation of ascorbic acid due to the advance of senescence and the stress due to water losses (Lee and Kader, 2000), it should be noted that although there were no significant differences in the concentration of vitamin C among fruits harvested from trees with HLB and asymptomatic, the latter had a higher concentration (Table 1); likewise, in both cases the concentration of ascorbic acid was between the ranges reported by other researchers with 39.3 mg 100 $g^{-1}$ (Zea-Hernández *et al*., 2016) and 45.2 mg 100$g^{-1}$ (Álvarez-Armenta *et al*., 2008) .

On the other hand, the content of citric acid and SST remained without significant changes between the fruits coming from trees with and without HLB, as well as depending on the storage time, remaining similar to those at the time of harvest. Regarding the technological index (<value <juice quality in the citrus industry), their behavior tended to decrease, in both groups of fruits, during storage and although the IT was higher in the fruits harvested from asymptomatic trees, observed differences with respect to those from trees with symptoms of HLB; IT values of 3.9 have been reported in Mexican lemon fruits at harvest time (Obeed and Harhash, 2006), being lower than those obtained in the present experiment.

**Storage at refrigeration temperature**

In fruits harvested from trees with and without HLB stored at 9 ±1 ºC for two weeks, no significant differences were observed, among them, in weight losses, which were 6.4 and 5.7%, being similar (5.5 ±0.7%) to those reported by Muñoz-Lazcano *et al*. (2011) to the same cooling conditions; same behavior was presented when transferring to room temperature (Table 2). Depending on the storage time, in both groups of fruits the weight losses increased significantly both to the refrigeration conditions and to the ambient temperature. In the IC, no significant differences were observed between the fruits from trees with HLB and asymptomatic to the two storage conditions, depending on the storage time, the fruits of both groups presented a IC without differences, with respect to the value at harvest time, at the refrigeration outlet, and with differences after four and eight days of exposure to the environment (Table 2).

**Table 2. Evaluation of some of the quality components of Mexican lemon fruits stored at 9 ±1 ºC for two weeks and then transferred to room temperature (20 ±2 ºC) for 4 and 8 days.**

| Storage (days) | PP (%) | AT (% acid citric) | SST (%) | Index technological | IC (1 000a/bL) | Acid ascorbic (mg 100 mL$^{-1}$) |
|---|---|---|---|---|---|---|
| C/HLB | | | | | | |
| Harvest | 0 d | 7.7 a | 9.2 a | 2.6 ab | -13.2 a | 39.1 a |
| Exit* | 6.4 c | 7.4 a | 8.8 a | 3.4 a | -10.5 ab | 31 b |
| 4 | 9.6 b | 7.4 a | 8.8 a | 3.2 a | -5.3 bc | 23.3 bc |
| 8 | 13.9 a | 7.5 a | 8.9 a | 1.8 b | -3.3 c | 16.4 c |
| S/HLB | | | | | | |
| Harvest | 0 d | 7.5 a | 9.2 a | 3.3 a | -13.3 a | 43.6 a |
| Exit* | 5.7 c | 7.4 a | 8.9 a | 3.6 a | -11.9 ab | 31.5 b |
| 4 | 8.7 b | 7.1 a | 8.5 a | 3.4 a | -6.6 bc | 21.3 bc |
| 8 | 13.6 a | 7.3 a | 8.7 a | 2.6 ab | -4.8 c | 17 c |

*= two weeks of refrigeration (9 ±1 ºC). Means with the same letter in each column are not significantly different according to the test Tukey ($p \leq 0.05$).

During storage at both temperatures, losses of ascorbic acid were observed as a result of the exposure time, mainly when transferred to the environment; however, no significant differences were observed between fruits harvested from trees with symptoms of HLB and asymptomatic. Regarding the titratable acidity and TSS variables, no significant changes were observed between both groups of fruits and also due to the effect of storage time, their values remaining similar to those at the time of harvest (Table 2). In both fruits harvested from trees with symptoms of HLB and asymptomatic, IT tended to increase during the refrigeration period to 9 ºC for two weeks with values of 3.4 and 3.6, respectively (Table 2), coinciding this response with results obtained by Obeed and Harhash, (2006); however, when transferring to room temperature, IT tended to decrease as storage time advanced. There were no significant differences in the IT value between the two groups of fruits. The increase in this variable initially suggests a concentration effect where the content of juice, SST and other compounds may be interrelated; likewise, the concept of better juice quality based on the higher IT value is limited, since other compounds such as aromas are involved in flavor.

At the refrigeration outlet, fruits harvested from trees with symptoms of HLB presented a cold damage index of 27.7%, while the asymptomatic 13.3%, in both cases of light character (<10% of the surface of the epicarp with symptoms of chopped). According to McDonald *et al*. (1993) the composition and morphology of epicuticular waxes influences the development of cold damage in citrus fruits, due to the alteration of the transport of gases and water vapor through the epicarp; although, in the present investigation, no differences were observed in weight loss (as an indicator of water loss), among fruits from trees with HLB and asymptomatic, in the former the tendency was for a greater loss, which would allow to assume that in the fruits with HLB the content and composition of episuticular waxes is affected, nevertheless this must be verified by other studies.

## Conclusions

At the time of harvest, there are no differences in quality, evaluated by the content of juice, SST, citric acid, ascorbic acid, technological index and color index, among fruits from trees with symptoms of HLB and asymptomatic.

The postharvest behavior of the fruits, in terms of weight loss, decrease in external green color, loss of vitamin C (ascorbic acid) and changes in the quality of the juice (IT), does not show differences between harvested trees with and without HLB, both at room temperature and refrigeration; however, in the fruits of trees with HLB said losses are more evident.

Fruits from trees with HLB are more sensitive to cold damage during storage at 9 ºC for two weeks.

It is suggested to carry out studies considering other parameters of quality such as content of functional compounds and physiological response by the application of superficial coatings, besides evaluating the quality of fruits coming from trees with symptoms of HLB, but with new production technologies.

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