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QUALITY AND NOVEL INDICATORS OF COMMERCIAL LIFE IN Valencia ORANGES (Citrus sinensis Osbeck)

NUEVOS INDICADORES DE VIDA COMERCIAL Y CALIDAD DE NARANJA cv Valencia (Citrus sinensis Osbeck)

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Abstract

Mexico is the fifth largest producer of fresh oranges in the world, and a new region in Morelos has recently become an important area of cultivation for cv Valencia. Even though fruit yields in this region are two times higher than the national average, fruit quality has not yet been adequately assessed. The objectives of this work were to assess fruit quality in the oranges from this region, and to determine their commercial life during storage. Significant differences occurred in the juice volume of fruits during all three seasons evaluated; however, no differences were detected in their weight, size, shape, rind thickness, or flavedo color (L*, C*, h). TSS/TA (total soluble solids/titratable acidity) ratios were high (10.3-12.9) in fruits collected during spring and summer. Overall, the oranges met the specifications of both national and international quality standards. Changes during storage indicated that compression force, % weight loss, and sensorial flavor were negatively impacted by storage at 24°C while TSS and TA of juice remained unaffected at 11 and 24°C. The commercial life of oranges was 21 d at 24°C and > 28 d at 11°C as determined from changes in the concentrations of certain aroma compounds found in the juice and from differences in the firmness of their rinds. Such changes correlated well with both weight loss and the sensorial perception of quality attributes. *Keywords*: preharvest factors, aroma compounds, compression test, sensory evaluation.

Resumen

México es el quinto productor de naranjas en el mundo y recientemente una nueva región en Morelos se convirtió en una importante área de cultivo. El rendimiento de fruta en esta región es dos veces mayor al promedio nacional pero su calidad no se ha caracterizado completamente. El objetivo de este trabajo fue medir la calidad de las naranjas cv Valencia de esta región y determinar su vida comercial durante el almacenamiento. En las tres temporadas de cosecha evaluadas se observaron diferencias significativas en el volumen de jugo de las frutas pero no en las variables: peso, tamaño, forma, grosor de la cáscara y color del flavedo (L*, C*, h). El cociente SST/AT (sólidos solubles totales/acidez titulable) fue mayor (10.3-12.9) en las cosechas de primavera y verano. En general, las naranjas cumplieron con las especifícaciones de calidad nacionales e internacionales. El almacenamiento de las naranjas a 24°C tuvo un efecto negativo en la firmeza, la pérdida de peso y el sabor, mientras que los SST y la AT del jugo no se vieron afectados durante el almacenamiento a 11 y 24°C. La vida comercial de las naranjas fue de 21 d a 24°C y >28 d a 11°C, de acuerdo con los cambios observados en las concentraciones de ciertos compuestos del aroma del jugo y la firmeza de sus cáscaras. Dichos cambios se correlacionaron bien con la pérdida de peso y la percepción sensorial de los atributos de calidad.

Palabras clave: factores precosecha, compuestos del aroma, pruebas de compresión, evaluación sensorial.

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1 Introduction

Apart from being one of the main exporters of citrus fruits, Mexico was in 2015 the fifth largest producer of fresh oranges after Brazil, China, India, and the United States (FAO, 2017). World production of this fruit is estimated to reach 49 million tons during 2017-2018. The latter, however, represents a 7.5 % decrease from the previous period (2016-2017) and is likely the result of unfavorable weather conditions in the major regions of production. Despite this, global fresh consumption (calculated at around 29 million tons will remain essentially the same as lower volumes are likely to affect only fruit processing. In contrast, Mexico's production of fresh oranges will probably remain unaltered, which could soon turn this country into the fourth largest producer in the world (USDA, 2018).

In Mexico, citrus fruits are commercially grown in 23 states, which combined have a total cultivated surface area of roughly 335 thousand ha. Approximately 63% of this surface is destined for the production of oranges, with most of it corresponding to the Valencia cultivar alone. National production of this fruit reached a total of 4.5 million ton during the 2014-2017 period, with per capita consumption calculated at 37 kg during 2017 (SAGARPA-SIAP, 2017).

Most orange plantations in Mexico are located within regions of humid and subhumid tropical climates (Medina *et al.*, 2007) and the state of Morelos is characterized by such conditions; however, orange orchards have only recently been established here. Additionally, practical information concerning their adequate agronomic management is generally lacking, as are studies that deal with the quality of the fruits produced under the type of ecological conditions that are particular to this region (Alia-Tejacal *et al.*, 2012). Despite these limitations, orange orchards continue to expand in this state, with yields that frequently double the national average (SAGARPA-SIAP, 2017).

Owing to their pleasant taste and high content of both nutritional and functional compounds, oranges are among the most consumed fruits in the world. Orange juice is, for example, rich in sugars, amino acids, minerals, and various types of organic acids. In addition, the pulp and rind contain several antioxidant compounds including vitamins C and E, carotenoids, and different kinds of polyphenols (e.g. flavonoids and hydroxycinnamic acids). Pectins and dietary fiber are also present, as are many volatiles responsible for the characteristic aroma of these fruits. As a result, orange consumption is thought to exert many beneficial effects on the overall health of humans (Rapisarda *et al.*, 2008; Hernandez-Carrillo *et al.*, 2015; Romero-Cano *et al.*, 2015; Goncalves, 2017).

Various climatic factors and cultural practices affect fruit quality and development, causing considerable variations in physical, chemical, and sensorial characteristics (Pelayo-Zaldívar, 2010). In the case of citrus fruits, the effects of temperature, relative humidity, mineral nutrition, and irrigation on either the chemical composition of their juice or on their external and internal morphological characteristics have all been fairly known for some time (Reuther, 1973; Ahmad *et al.*, 2013). For instance, warm and dry climates tend to produce large and elongated oranges with thick and rough rinds, whereas night temperatures above 13°C produce internally mature fruit with green flavedos (Reuther, 1973).

Despite such variations in their morphology and chemical composition, fresh oranges must meet a minimum set of quality standards in order to be deemed suitable for commercialization. In this sense, a good maturity (or harvest) index to follow is a juice TSS/TA ratio \geq 8.0, together with the presence of a yellow-orange color in at least 25% of the rind surface (Arpaia and Kader, 2012). Other important quality attributes are the size and shape of the fruit, their juice volume, firmness, and rind smoothness.

Flavor quality, on the other hand, is generally evaluated by taking into account the TSS/TA ratio of their juice, the presence and quantity of volatiles responsible for a "fresh" orange aroma, and the absence of any off-flavors and scents. In this context, only a few key volatiles have any practical significance. For instance, even though freshlysqueezed orange juice contains more than 300 aroma compounds, only 25 are reported to have important odoriferous activities (Rouseff, et al., 2009). In fact, the volatiles ethyl butanoate, linalool, β -mircene, octanal, and decanal are, in essence, the main contributors to the "fresh" orange aroma of recentlysqueezed juice (Ahmed et al., 1978; Hinterholzer and Schieberle, 1998; Pérez-Cacho and Rouseff, 2008). There exist, on the other hand, certain volatiles associated with an "old" orange odor which must be avoided whenever possible. These are generally formed as fruits undergo senescence. The alcohol α terpineol, for example, is a well-known contributor to the off-flavor present in both fresh and processed

orange juice (Pérez *et al.*, 2005; Qiao *et al.*, 2008). All three flavor components (aroma volatiles, sugars in TSS, and organic acids in TA) can be evaluated by chemical and sensorial methods. As examples of the latter, sensory evaluations can be practical ways to assess such changes. In addition, they can also be used as guidelines for the establishment of commercial life in fresh produce.

Despite the fact that oranges have been the focus of intensive postharvest research, there are no reliable indicators from which to predict the time at which these fruits have completely exhausted their commercial life. In addition, aroma compounds and textural properties have not yet been used as objective indicators of storage potential, nor has sensory evaluation been explored as an alternative for the establishment of postharvest life. With this in mind, we first decided to investigate the effect that different harvesting seasons had on the quality of Valencia oranges as they are currently produced in a newlyestablished region of cultivation in the municipality of Tepalcingo (state of Morelos), Mexico. Next, we investigated the usefulness of different parameters, namely firmness, aroma volatiles, and sensory attributes, in the assessment of postharvest life in these fruits.

2 Materials and methods

2.1 Plant materials

Valencia oranges (Citrus sinensis) were harvested from a commercial orchard located in the municipality of Tepalcingo (close to the town of Zacapalco) in the state of Morelos, Mexico (see Fig. 1). The orchard is known as "Campo el Pochotillo", and at the time of our study, had the following characteristics: 1) a clay loam soil with a pH=8.1; 2) 10 to 11-year-old Valencia trees grafted onto C. volkameriana rootstocks; 3) drip irrigation (480 l/h) applied every 8 d for 48 h; 4) flowering cycles occurring from February to March and from June to July); 5) natural pollination mediated by bees; and 6) fruit production averaging 40 ton/ha. Additionally, two different fertilization schemes were used each year. The first one was applied during the rainy season (1.2-0.4-0.2 kg of N-P-K per tree) when the foliage was still growing; the second one during the dry season and consisted of an addition of urea (46-00-00) in combination with irrigation.



Fig. 1. Location of the orchard known as "Campo el Pochotillo" in the municipality of Tepalcingo, state of Morelos, Mexico (Coordinates: LN 18°38'69", LW 98°559'6.9"; Altitude: 1 086 m above sea level).

In order to evaluate the effects that local environmental conditions had on fruit quality, every month a selection of 20 oranges was harvested from four different Valencia trees. The latter took place during the 2012 spring (April-June), summer (July-September), and autumn (October-December) seasons of production. Trees were selected at random, and the fruits were always harvested at commercial maturity (i.e. 248 d from anthesis, when their flavedo was vellow-orange and their juice TSS = 8.5-11.6% [10%] on average]). After harvesting, the following variables were measured: weight, rind thickness, flavedo color (as determined from the L*, a*, and b* color coordinates), polar and equatorial diameters (PD and ED), and fruit shape (as determined from the PD/ED ratio). Additionally, the following were also measured in juice: total soluble solids (TSS), titratable acidity (TA), and total volume (% w/w).

In order to monitor any postharvest changes in fruit quality, a separate sample of oranges was collected during the month of February (2014). The fruits were harvested using the same criteria as before and were selected based on the absence of defects. They were then randomly grouped into different experimental units before being stored at either 11 or 24°C with no control over relative humidity (RH) but average values of 75 and 59%, respectively. Each experimental unit consisted of six fruits, with three repetitions being performed for every temperature and time. Throughout storage, different variables related to fruit quality and sensorial attributes were measured.

2.2 Quality assessment

Rind color was measured using a Hunter-Lab ColorFlex Colorimeter (Reston, USA). For the latter, two equatorial regions on each fruit were selected, and the L*, a*, and b* color coordinates were determined (Raddatz-Mota *et al.*, 2016). Using these data, the values of C* and h, together with the citrus color index (CCI), were then calculated according to the following equations: $C* = [(a*)^2 + (b)^2]^{1/2}$, $h = \tan^{-1}[a*/b*]$ (Konica Minolta, 1998), and CCI=1000·a*/L*·b* (Artés *et al.*, 2000; Pérez-Grijalva *et al.*, 2018).

Firmness was determined on whole fruits by means of a compression test. For this, a Brookfield Texture Analyzer C3 (Brookfield AMETEK, MA, USA) was used. The instrument was equipped with a cylindrical probe that delivered 4N of force at a speed of 1mm/s. All measurements were made at the equatorial side of each fruit. During these determinations, the force necessary for the non-destructive compression of the rind to a depth of 5 mm from the initial point of contact was recorded and expressed in Newtons (N).

Composite juice samples from each experimental unit (i.e. group of six oranges) were extracted using a Philips HR2737 Comfort® citrus press juicer. These were then frozen in liquid nitrogen and stored at -70°C until analysis. The content of TSS, TA, and of five major aroma compounds (ethyl butanoate; 3,7-dimethyl-1,6-octadiene-3-ol, also known as linalool; 1-methyl-4-(1-methylethenyl)-cyclohexene, also known as limonene; α -terpineol; and 4-ethenyl-2-methoxyphenol, also known as p-vinylguaiacol) was subsequently determined in thawed samples. TSS values were obtained as percentages using an Atago N-1 alfa hand-held refractometer (Atago Co. Ltd., Tokyo, Japan). TA was calculated from titration against 0.1 M NaOH and the results expressed as percentages of citric acid as this is the predominant organic acid in oranges. The maturity index (TSS/TA ratio) was then calculated from the values of TSS and of TA that were obtained. Finally, any changes in fruit weight resulting from transpiration were measured in six different experimental units. This was done over the course of storage at either temperature (11 and 24°C) using an Ohauss balance and results were reported as % weight loss.

2.3 Analyses of volatile aroma compounds

For these determinations, orange rinds were separated manually in order to avoid mixing the aroma volatiles from the juice with those present in the essential oils of the rind. The analysis itself was performed according to the procedure outlined by Díaz de León et al. (2009). In order to avoid the formation of chemical artifacts derived from oxidation and/or other enzymatic reactions, 1 mL of an EDTA-NaOH solution (0.55 M EDTA; 1.66 M NaOH) was added to every 20 mL of juice to be analyzed. These were then frozen in liquid nitrogen and stored at -70°C until analysis. For the quantification of volatiles, 3 mL aliquots of the defrosted juice were individually placed inside 10 mL vials containing 1.2 g of NaCl (because of the salting-out effect, the NaOH helps release volatiles from the juice matrix into the headspace air). The vials were then covered with black Viton septa and Teflon silicone rings, before being crimp-sealed using aluminum rings. Immediately after, the vials were agitated for one min using a vortex, equilibrated at 30°C in a thermoblock, and a headspace sample taken by inserting a 50/30 µm DVB-CAR-PDMS Solid Phase Microextraction (SPME) fiber (Supelco, Bellefonte, PA, USA). To ensure the adequate adsorption of volatiles to the fiber, the latter was exposed for 10 min to the headspace air from each vial. The compounds were then desorbed at 250°C for 10 min inside the injection port of a Hewlett Packard 5800 series II gas chromatograph (Palo Alto, USA) set to operate in "splitless" mode using a purge time of 3 min. The GC had previously been equipped with a DB-WAXETR capillary column (0.32 mm x 60 m, 1 μ m film thickness; J&W Scientific, Folsom, USA) and a FID detector. Both injector and detector temperatures were set at 250°C, and in the case of the column, the following program was applied: an initial temperature of 60°C for 5 min, followed by a 5°C/ min increase to 230°C for 7 min. The ppm concentration of the compounds ethyl butanoate, limonene, linalool, α -terpineol, and p-vinylguaiacol was obtained from curves prepared using commercial standards. For these curves, the compounds were first dissolved in a simulated orange juice as reported by Peleg *et al.* (1992) before being treated to the same chromatographic conditions as those that would be used during the quantification of actual samples. Compound identities were further verified using GC-MS (HP 6890-5975B, 4 scans s⁻¹ mode, 18-500 m/z range, 70 eV ionization energy), the NBS75KL library, and known chemical standards.

2.4 Sensory evaluation

Level of pleasantness sensory tests were carried out on oranges that had been stored for 0, 7, 14, and 21 days at either 11 or 24°C. For this, a group of 45 panelists was asked to rate the appearance, odor, and taste (i.e. flavor) of the fruits that were presented to them by using a seven-point hedonic scale which ranged from 7 = "I like it a lot" to 1 = "I dislike it a lot". They were additionally asked to grade firmness and freshness according to the five-point "Just About Right" (JAR) scale (which ranges from 5= "Much more than I like" or "Much more than I expected" to 1= "Much less than I like" or "Much less than I expected"). This scale was adapted for use in Spanish, with the central point lying in 3= " just as I like". The last two quality attributes (i.e. firmness and freshness) were selected because they are the two most relevant parameters influencing consumer preference in oranges. The latter was concluded based on the results of a preliminary sensory evaluation which was conducted in order to optimize the design of the aforementioned tests.

2.5 Statistical analysis

NCSS software (Kaysville, UT, 2000) was used for all statistical analyses. First, averages and confidence intervals were calculated in order to determine the effect that local environmental conditions had on orange quality. Then, an ANOVA analysis was performed so as to accurately discern the effect of temperature and storage time on the physical and chemical characteristics of fruits. Tukey's multiple comparison test (α = 0.05) was performed whenever significant differences were detected. WL data were analyzed independently as they were not dependent on initial values. In the case of sensory evaluations, a frequency distribution analysis was performed for data concerning firmness and freshness.

3 Results and discussion

3.1 Effect of environmental conditions on fruit quality

It is well documented that temperature has a significant effect on the weight and shape of oranges (Reuther, 1973; Albrigo, 2010; Sinclair, 2012). However, we failed to find any large variation in either of these two parameters over the course of our study (i.e., the spring, summer, and autumn seasons of production, see Table 1). The latter is most likely the result of the prevailing climatic conditions in this region of Morelos, which is characterized by stable warm temperatures with very little variation year-round (avg. yearly temperature=22°C, avg. temperature of coldest month=18°C; Ornelas et al., 1990). Average fruit weights (245.7-256.1 g, all three seasons included) were also quite similar to those of oranges gathered from several nearby orchards (240-275 g and 240-301 g, avg.=260 g; Alia et al., 2009; Ariza et al., 2010) and the EDs of our fruit (75-80 mm) frequently complied with calibration code No. 4 of the Mexican quality standard (NOM-NMX-FF-027-SCFI-2007, SAGARPA 2007). This made our oranges suitable for commercialization, at least in this one regard.

Table 1. Fruit and juice characteristics of Valencia oranges harvested over the course of three different harvesting seasons.

Harvesting season		Juice characteristics								
	Weight (g)	Shape (PD/ED)	Rind thickness (mm)	L*	C*	h*	Volume (% w/w)	TSS	ТА	TSS/TA
Ap-Jun	245.7a	0.96a	6.2a	62.5a	56.2a	90.6a	52.8a	11.4a	0.9a	12.9a
Jul-Sep	248.2a	0.99a	6.3a	66.7a	55.6a	92.2a	55.4ab	10.8a	1.1ab	10.3a
Oct-Dic	256.1a	1.03a	5.6a	70.5a	61.1a	91.6a	59.4b	9.5b	1.5b	6.4a

PD/ED=polar diameter/equatorial diameter. Values are the means of monthly averages obtained over the course of each harvesting season. Values with different letters in the same column are significantly different (95% confidence interval).

Fruit texture can likewise be influenced by the environment (e.g. light, temperature, and soil moisture; Sams, 1999). Rind thickness and smoothness are, for instance, strongly affected by both temperature and RH - especially during fruit development (Reuther, 1973; Sinclair, 2012). Although, there is currently no definitive range of values from which to classify rinds as either thick or thin, those present in our fruit are perhaps best described as thick (season averages of 5.6-6.3 mm) according to the precepts followed by certain authors (Reuther, 1973; Ariza et al., 2010). Nevertheless, just as occurred with weights and PD/ED ratios (i.e. fruits shape), we failed to find either large or statistically significant differences in this parameter over the course of our study (i.e. all three seasons of production, see Table 1). It appears, therefore, that both temperature and RH varied little throughout most of the growth period. Additionally, foliar analysis revealed optimum levels of NPK (21.3, 2.2, and 16.0 g/kg, respectively) to be present in trees, suggesting that proper agronomic management was followed (Transito, 2013). The latter is relevant because excess potassium will often result in larger and coarser fruits, just as phosphorous deficiencies can lead to oranges with noticeably thicker rinds (Ladanyia and Ladinaya, 2010).

Another factor that is influenced by both climatic conditions and agronomic practices is rind color (Agustí-Fonfría, 2012; Soler-Aznar and Soler-Fayos, 2006). For instance, warm climates with very little temperature variations between day and night will often result in internally mature oranges with green flavedos (Reuther, 1973; Sinclair, 2012). Likewise, the application of fertilizers that contain high levels of nitrogen can lead to oranges with higher proportions of green on their surface (Koo and Reese, 1977; Romojaro, et al., 2003). In this context, our results appear to suggest that oranges from Tepalcingo must have frequently been exposed to large, periodic variations between day and night temperatures as normal color development occurred during all three season of production. For the same reason, and considering also the results of our foliar analysis, nitrogen fertilization was likely never an issue either.

The Mexican quality standard known as NOM-NMX-FF-027-SCFI-2007 (SAGARPA, 2007) specifies that the juice volume of oranges should never fall below 40% (w/w), whereas the international CODEX STAN 245 (FAO, 2004) sets this minimum at 35% (w/w). In our study, the juice volume of oranges

not only met both requirements during all three seasons of production, but additionally experienced a significant increase (α =0.05) in fruits harvested during autumn compared with those harvested during spring (Table 1).

Another important quality attribute is the TSS/TA ratio, which in oranges must either be ≥ 7.0 (NOM-NMX-FF-027-SCFI-2007) or otherwise span the interval 7.0-10.0 as specified by several international standards (like those of Florida, California, and Arizona in the USA, as well as that of Australia). At Tepalcingo, all oranges harvested during the spring and summer seasons had higher TSS/TA ratios (10.3-12.9) than the minimum set by any of these norms. However, those harvested during autumn tended to have much greater acidities (TA=1.5%), and as a result, exhibited lower TSS/TA ratios on average (TSS/TA=6.4, see Table 1). This contrasts with previous studies of nearby orchards, where higher TSS/TA ratios (TSS/TA=9.9-15.7; TA=0.66-1.00%), were consistently reported during all seasons examined (Ariza et al., 2010). Nevertheless, it can still be concluded that oranges of an acceptable quality can easily be produced under the prevailing climatic conditions of this region of Morelos (characterized by a warm subhumid environment), and that several quality characteristics repeatedly meet and/or exceed both national (i.e. Mexican) and international standards, making these fruits suitable for commercialization.

3.2 Visual and chemical changes during storage

3.2.1 Fruit and juice quality characteristics

Rind color was previously discussed to have developed normally, likely as a result of the large, periodic variations between day and night temperatures in this region of Morelos. However, in order to more accurately assess this parameter (particularly for commercial purposes), the Citrus Color Index (CCI) was calculated using the L*, a*, and b* coordinates of stored oranges. The CCI is used in the citrus industry in order to determine such things as: 1) the optimal date for harvest; 2) the maturity stage at which the fruits arrive at packing facilities, and 3) if said fruits should first undergo degreening, or are otherwise apt for commercialization (Vidal et al., 2015). Besides being practical, this index also correlates strongly with both the visual appreciation of color by consumers and their specific level of preference (Artés et al., 2000).

	Storage days	TSS%	TA%	SST/AT	% Juice	Flavedo color				Juice color				
ane														
Storage temperatu						L*	a*	b*	ССІ	L*	а*	b*	CCI	
Initial	0	11.27	0.742	15.1	54.00	78.64	15.96	75.43	2.69	31.40	0.74	20.72	1.13	
11ºC	7	11.27	0.666	16.99	43.80*	77.69	18.01	71.75*	3.22	31.44	2.12*	22.42	2.96*	
	14	11.13	0.755	14.76	47.66	78.18	18.17	72.87	3.19	33.46*	1.82	24.01*	2.12	
	21	11.20	0.713	15.75	56.82	77.16	21.15*	73.27	3.78	31.49	1.28	23.56*	1.71	
	28	11.00	0.832	13.28	48.34	79.85	23.05*	75.07	3.86*	29.93	0.80	22.87	1.10	
24°C	7	10.87	0.708	15.42	47.98	78.05	14.09	72.03*	2.49	33.42*	1.62	22.25*	2.16	
	14	11.40	0.751	15.20	50.13	77.83	18.13	72.56	3.19	31.79	0.83	22.02	1.18	
	21	11.60	0.777	15.05	56.38	78.87	22.00*	75.04	3.72*	30.55*	0.36	21.60	0.50	
	28	11.53	0.713	16.22	54.46	76.16*	27.15*	72.15	5.01*	29.95*	0.74	22.64*	1.09	

Table 2. Fruit and juice characteristics of Valencia oranges harvested in February and stored at 11 and 24 °C.

Values are the means of three repetitions. Values followed by an asterisk in the same column are significantly different compared to initial values (0 d) according to Tukey's multiple comparison of means ($\alpha = 0.05$).

For instance, a CCI > 6 indicates the presence of an intense yellow-orange color (which is usually the tone preferred by most consumers) whereas a value < 6 but > 0 only denotes the predominance of yellow. On the other hand, a CCI < 0 strongly implies the prevalence of green, something which is highly undesirable for oranges (Martínez-Jávega et al., 2004). In this context, the CCI of our fruits (i.e. those harvested during February) ranged from 2.7-3.9 and from 2.5-5.0 when kept at 11°C and 24°C, respectively. In fact, the CCI of both treatments increased significantly towards the end of storage (although this change was perhaps more noticeable in oranges kept at 24°C). Collectively, our results not only suggest the predominance of yellow in the rinds of these fruits, but that subsequent increments in the intensity of this color are apparently unimpeded by storage at 11 or 24°C (Table 2). Given that both Mexican and international standards merely specify that oranges "must present the characteristic color of their variety", the fruit from Tepalcingo can easily be said to comply with this requirement. The CCI of their juice, on the other hand, showed no significant differences with respect to values determined at the beginning of storage, save for those obtained on d 7 at 11°C (Table 2). Thus, juice color is also largely unaffected by storage time or temperature.

After storage for 28 d, no significant differences (α =0.05) were detected in the TSS content of fruits, nor in their values of TA, TSS/TA, and juice volume. The latter occurred irrespective of storage temperature (11 or 24°C) and whenever final values were compared with those determined at the beginning of storage (Table 2). Additionally, the TSS/TA ratio remained well above the 7.0 minimum specified by the Mexican standard or the 7.0-10.0 interval set by the standards of other regions and countries (e.g. Florida, California, Arizona [in the USA], and Australia) (Ariza *et al.*, 2010). Similarly, the juice volume of oranges never fell below the recommended levels established by either national (40%, NOM-NMX-FF-027-SCFI-2007) or international (35%, CODEX STAN 245) norms.



Fig. 2. Weight loss in Valencia oranges during storage at 11 and 24 °C. Values are the means of six repetitions \pm SD.

3.2.2 Weight loss

After harvesting, storage can generate considerable problems for many fruit crops including oranges. Prominent among them is the issue of weight loss (WL), which is mainly the result of transpiration. In this case, a difference in the external pressure of water vapor propitiates its escape through various epidermal structures on the fruit surface (e.g. stomata, lenticels, and the cuticle) (Kader, 2002). As a result, fruit quality can become seriously compromised (e.g. loss of turgor and of an overall "fresh" appearance) which will often lead to a generalized lowering of prices. Finally, WL can itself depend on the storage temperature/RH that is applied, as ambient conditions will usually result in moisture loss that is several times higher than the one observed during low-temperature storage (Kader, 2002).

As expected, WL was, in this study, strongly influenced by the conditions of storage, being more pronounced at 24°C and 59% RH than at 11°C and 75% RH (Fig. 2). After 14 d, WL was 10.6% at 24°C (i.e. close to the limit of commercial tolerance, which is 10% according to most producers) and 4.2% at 11°C; however, after 28 d, the values had increased to 21% at 24°C and to 10% at 11°C (Fig 2). This indicates that after two weeks of storage at ambient temperature, WL would be roughly equivalent to 100 kg/ton of fruit. This is noticeably different from the 40 kg/ton of fruit that would only be lost during storage at 11°C. If, on the other hand, the storage periods were to be doubled, WL would be equivalent to 210 kg/ton of fruit at 24°C compared with 100 kg/ton of fruit at 11°C.



Fig. 3. Firmness in Valencia oranges stored at 11 and 24 °C. Values are the means of three repetitions. Among the same storage temperatures, data points followed by the same letter are not significantly different, whereas those marked with an asterisk are statistically significant (Tukey's multiple comparison of means, $\alpha = 0.05$) compared with the initial value obtained at d 0.

Generally speaking, WL was 2.5 times higher at 24°C than at 11°C even after 28 d of storage.

3.2.3 Compression tests on whole fruits

In oranges from Tepalcingo, firmness correlated well with WL, and both of these were strongly dependent on storage times. For instance, in fruit kept at 24°C, the force necessary to compress the rind by a total of 5 mm decreased significantly during the first two weeks (from 35 to 28 N, see Fig 3); however, as weight loss progressed, the magnitude of this force eventually began to increase (changing, for example, from 28 N on d 14 to 34 N on d 21). Ultimately, a point was reached where the rind became quite leathery as a result of dehydration (roughly corresponding to the 50 N force obtained on d 28). In this context, a good indicator that the commercial life of oranges has probably been exhausted is the inflection point observed on d 21, which is later followed by a rapid increase in fruit firmness (Fig. 3). That such an inflection point does not occur in the graph of fruit kept at 11°C, even after 28 d, demonstrates the effectiveness of low-temperature storage in delaying such changes. Nevertheless, in a study with cv Nagpur Mandarin, Singh and Reddy (2006) failed to find a similar inflection point in the firmness graph of fruit kept at either low (7°C) or ambient (28°C) temperature (corresponding to 78 and 58% RH, respectively).



Fig. 4. Aroma volatiles detected in the juice of Valencia oranges when stored at 11 and 24 ?C. A) Limonene; B) Ethyl butanoate; C) Linalool; D) α -terpineol. Values are the means of three repetitions. Among the same storage temperatures, data points followed by the same letter are not significantly different, whereas those marked with an asterisk are statistically significant (Tukey's test, $\alpha = 0.05$) compared with the value at d 0. Levels of linalool and ?-terpineol were far too small to be accurately measured by either of our standard curves, thus they are reported simply as <0.01ppm and presented only as chromatogram areas.

This, however, could simply be due to the relatively short time frame used to evaluate the oranges (barely 10 d in total). Despite this, the authors did find that compression force decreased as storage times increased, and that this change occurred much faster and was of a greater magnitude in fruit kept at 28°C.

3.2.4 Sensory evaluation

Textural changes are important to consider as they strongly influence consumer acceptance (Valero y Serrano, 2010). In our study, both groups of oranges (i.e. those stored at 11 and 24°C) were evaluated sensorially for their level of firmness by a group of 45 panelists; overall, their responses agreed with the results of our compression tests. For instance, coinciding with the inflection point on d 21, a majority the panelists agreed that oranges stored at ambient temperature were "harder than I like". On the other hand, most panelists considered refrigerated oranges to be "firm, neither hard not watery", and regarded them as such for the duration of the experiment. Although firmness in these fruits did decrease somewhat as a result of low-temperature storage (just as described previously in section 3.2.3, see Fig 3), this change was slow and not too large in magnitude, and at any rate, ceased altogether during the last few days.

In terms of firmness, most panelists agreed that oranges stored at ambient temperature could be adequately preserved for 7 d only ("Firm, neither hard nor watery"); afterwards, they were considered to be either too watery ("More watery than I like") or too hard ("Harder than I like") depending on storage time (d 14 and 21 respectively, see Fig. 6A). By comparison, refrigerated fruit remained firm even after 3 weeks (Fig. 6B). Similarly, fruits kept at ambient temperature were considered to be optimally fresh ("Fresh, just as I like") during the first few days of storage. By d 14 however, most panelists felt inclined to describe such fruits as being "Less fresh than I like" (Fig. 6C). Refrigerated fruits, on the other hand, were considered to be adequate (i.e. "Fresh, just as I like") for the entire duration of the experiment. Additionally, the number of panelists labeling fruit with this descriptor increased to 71% after 21 d (Fig. 6D).



Fig. 5. Appearance and flavor of Valencia oranges stored at 11 and 24 °C. Average of ratings assigned by 45 panelists. Means followed by the same small letter are not significantly different among storage days, whereas those followed by the same capital letter are not significantly different compared to the initial value (7 d).

3.2.5 Aroma compounds in juice

In citrus fruits, aroma compounds can be found either in the rind (inside the glands of the essential oils) or in the juice vesicles themselves (within the oil sacs usually found therein). Typically, their composition varies depending on the tissue in question, although this can also depend on the particular method used for their extraction (Baldwin *et al.*, 2012; Bai *et al.*, 2016). Special care must therefore be taken during sample collection in order to prevent such compounds from migrating between different tissues and potentially altering results (Perez-Cacho and Rouseff, 2008). With this in mind, the oranges in this study were first peeled manually before their juice was extracted (i.e. to prevent contamination with rind volatiles).

Our results indicated that limonene (the most abundant monoterpene in orange juice but one with almost no influence on its flavor) was not significantly affected by storage until after d 14, when it began to decline significantly (α =0.05) regardless of temperature (Fig. 4A). On the other hand (and after roughly the same time period), the concentrations of α -terpineol increased noticeably in fruit stored at ambient temperature (24°C), reaching significantly higher levels on d 21 compared with those found on d 0. This trend continued to hold true until the very end of storage (Fig. 4D). Results like these suggest that limonene may indeed serve as a type of precursor during the synthesis of α -terpineol, just as was proposed by Haleva-Toledo et al. (1999) and Pérez-López et al. (2006) during the storage of processed orange juice. Reinforcing this notion is the fact that, in mandarins stored at 20°C, the concentrations of dlimonene decline to 80% after just 9 d (from 34,516 to 28,416 ng/mL juice), while those of its oxidation product (i.e. α -terpineol) may rise to 300% (from 197 to 605 ng/mL) after a comparable period of time (Pérez *et al.*, 2005). Finally, even though the levels of α -terpineol had, by d 7, increased significantly (α =0.05) in the juice of refrigerated oranges, they later dropped to levels similar to those found at the beginning of storage (on d 14), and remained there for the duration of the experiment. This result is important as α -terpineol is a monoterpenic alcohol associated with the aroma of "old" oranges, and its synthesis in stored fruits is therefore highly undesirable.

p-vinilguayacol, another volatile with limonene as a precursor in processed orange juice (Haleva-Toledo *et al*, 1999; Hiramoto *et al.*, 1999), was conspicuously absent from the juice of fresh fruits (0 d of storage) as well as from those of stored oranges (11 and 24°C). It is therefore likely that the synthesis of α -terpineol was preferred over the synthesis of this compound in the vesicles of our fruit. Like α -terpineol, p-vinilguayacol is also associated with the aroma of "old" oranges.

Ethyl butanoate, on the other hand, was present but decreased significantly (α =0.05) in fruits stored at either temperature (11 and 24°C); this decline, however, was noticeably more pronounced in refrigerated oranges (Fig. 4B). Ethyl butanoate is among the most abundant esters in these fruits and is closely associated with the "fresh" aroma of their juice (Brat *et al.*, 2003).



Fig. 6. Firmness and freshness of Valencia oranges stored at low and ambient temperature. Percentage of ratings assigned by 45 panelists. A and $C = 24 \text{ }^{\circ}C$; B and $D = 11 \text{ }^{\circ}C$

In contrast, the levels of linalool - a terpenic alcohol also associated with the aroma of "fresh" oranges - increased significantly after 7 d of storage $(\alpha=0.05)$ and remained at these levels for an additional week (i.e. until d 14). The latter remained true regardless of the conditions used (11°C vs. 24°C). Its levels later decreased, however, to values similar to those found at the beginning of storage (Fig. 4C). This coincided well with the aforementioned increase in the levels of α -terpineol. The changes in both of these volatiles can be explained by the fact that linalool is another precursor of α -terpineol (Haleva-Toledo et al., 1999). Supporting this are the results obtained by Pérez-López et al., (2006) in processed mandarin juice, where the formation of α -terpineol and terpinen-4-ol (both off-flavor components) coincided with the decomposition of d-limonene and linalool.

It appears, therefore, that the aroma of oranges progressively deteriorates over the course of senescence, especially when they are kept at room temperature (i.e. 24°C). Their TSS and TA, however, remain constant as neither of these undergo significant changes during storage (Table 2). The latter is noteworthy as TSS and TA are both major components of flavor. Refrigerated oranges, on the other hand, fare much better as the time-dependent natural variation normally observed in certain volatiles (e.g. α -terpineol) becomes significantly delayed at 11°C. The high scores assigned by the panelists to the juice of these fruits appear to confirm this (Fig. 5B). Low temperature, however, does have a negative effect on the synthesis of a few desirable compounds (e.g. ethyl butanoate, Fig. 4B), just as occurs in many other fruits such as the strawberry (Pelayo, *et al.* 2003) and the tomato (Díaz de León *et al.*, 2009).

Such changes can, on the other hand, be good indicators of stress. For instance, variations in the emission of certain volatiles often occur as a result of chilling injury (Obenland *et al.*, 2003), the application of postharvest thermal treatments (Pérez *et al.*, 2005), or as part of the normal process of senescence (Bai *et al.*, 2010; Hui, *et al.*, 2010). In this study, stress

was probably induced by the actions of harvesting and postharvest handling. Evidence for this comes from the fact that linalool increased after 7 d of storage, regardless of the actual temperature applied (Fig. 4C). In fact, high levels of linalool could still be detected after a further 7 d had elapsed (i.e. on d 14). Previous studies have similarly reported an increase in this compound as a result of damage caused by mechanical injury (Piesik *et al.*, 2006). It is also not uncommon for such stress responses to remain active for days or even weeks after the initial damage (Pantastico, 1979; Scherrer-Montero, 2011; Chamhum Salomão, 2014).

Conclusions

The quality of Valencia oranges from Tepalcingo (as evaluated over the course of three different harvesting seasons) metal specifications listed in both Mexican (NOM-NMX-FF-027-SCFI-2007) and international (Codex STAN 245) norms. The parameters evaluated included fruit shape, dimensions, TSS/TA ratio, juice volume, and CCI. Firmness (as determined from compression tests) was a good indicator of the commercial life of these oranges and correlated well with both weight loss and the level of consumer acceptance of several quality attributes (e.g. appearance, flavor, firmness, and freshness). A decrease in the concentration of ethyl butanoate (a compound associated with the aroma of "fresh" oranges) also occurred, as did an increase in the levels of α -terpineol. Both were especially informative of the progress of senescence in Valencia oranges. Based on all these findings, their storage life was determined to be 21 d at 24°C and > 28 d at 11°C.

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