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Research Paper

# Examining the impact of dry climates temperature on citrus fruit internal ripening

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A R T I C L E I N F O

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A B S T R A C T

High temperatures alter the ripening process of citrus fruit, affecting quality, flavor, harvest time and marketing period. For example, citrus fruits ripen faster and are sweeter in tropical hot-humid climates than in Mediterranean warm-dry climates due to higher sugar accumulation and organic acid catabolism. Considering that: 1) over 60 % of the world's citrus exports come from countries with warm Mediterranean climates, and 2) the Mediterranean region is warming 20 % faster than the global average, the citrus industry will face significant challenges in the coming years. The range of high temperatures and the timing at which they determine sugar and acid content in dry climates have not been studied under field conditions. Therefore, a study was undertaken over 6 years to determine the relationship between high temperature and citrus ripening in two dry macroclimates, hot (Arid, in Morocco) and warm (Mediterranean, in Spain), and 5 microclimates of the Mediterranean region.

Heat stress in these dry climates correlates with changes in fruit quality (lower juice and sugar content, and higher organic acids), depending on the maximum temperature threshold and the time of onset and duration of heat. In the arid climate, the heat stress threshold was found to be a significant number of days above 35 °C. This was particularly important at the beginning of summer (July in the northern hemisphere). However, in the current Mediterranean climate of Spain, the percentage of summer days above 35 °C is still low indicating that other microclimatic conditions are involved in determining fruit quality. In particular, an increase in the number of days with temperatures between 27 °C and 33 °C in late summer and early autumn was found to correlate with reduced citric acid concentration. Microclimate did not determine sucrose, fructose, and glucose concentrations.

## 1. Introduction

Climate plays an important role in fruit development and ripening, as metabolic processes such as fruit color (Mesejo et al., 2022) or acidity (Saini et al., 2019) are temperature dependent. This is particularly important for *Citrus* species because, although they originate from subtropical climates where ripening occurs during the cool season, they are cultivated in many climates around the world where fruit quality varies considerably: three tropical climates (Af, Am and Aw), four arid climates (BWh, BWk, BSh and BSk) and six temperate climates (Csa, Csb, Cwa, Cwb, Cfa, Cfb), according to the Köppen-Geiger climate classification (Peel et al., 2007). The internal citrus fruit quality, i.e. oranges and mandarins, is defined at stages II and III of fruit development. During Stage II (summer) the fruit accumulates water and carbohydrates and synthesizes organic acids. At the end of Stage II and during Stage III (autumn), the concentration of carbohydrates continues to increase while the concentration of acids decreases, partly by catabolism and partly by dilution (Cercós et al., 2006). At this stage, pigments also accumulate in the peel and the pulp (Alquézar et al., 2008). The concentration of carbohydrates is not significantly reduced and represents 80 % of the total soluble solids (TSS), while citric acid is the main organic acid (Cercós et al., 2006).

The most comprehensive study on the influence of macroclimate on the internal ripening of orange fruit compared tropical and temperate climates, specifically the hot humid climate of Colombia (Af and Aw) with the warm dry climate (Csa) of California in the United States (Reuther and Rios-Castaño, 1969). In tropical conditions, with constantly high temperatures and high humidity, the fruit sugar content

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increases, and the acid content decreases compared to the Mediterranean climate (Csa), with dry summer and high day/night temperature variations. This field study does not allow the effect of temperature to be separated from the effect of humidity (humid vs. dry climate). Therefore, in this study the role of temperature in fruit ripening was assessed by comparing two dry macroclimates: the arid climate (BSh) and the Mediterranean climate (Csa).

Temperature is generally considered to be negatively correlated with acidity in citrus (Iglesias et al., 2007; Lado et al., 2018; Sadka et al., 2019; Saini et al., 2019; Kim et al., 2021). In fact, under greenhouse conditions an increase in day and night temperature reduces citric acid concentration (Kim et al., 2021). In particular, the decrease in acidity due to temperature increase is explained by a decrease in malate and citrate concentrations, as the activity of enzymes contributing to glycolysis and the TCA cycle is modified (Etienne et al., 2013; Hussain et al., 2017). The relationship between high temperature and TSS content in citrus is unclear, as direct, inverse, and no relationships have been reported (Reuther and Rios-Castaño, 1969; Saini et al., 2019; Kim et al., 2021)

Citrus acid concentration is also reduced by the increase in fruit volume during fruit growth increase, so water relations should also be considered. In fact, regulated deficit irrigation during phase II (the acid synthesis phase) reduces juice content and increases fruit acidity at harvest in the Csa climate (Ballester et al., 2011, 2013, 2014; Pérez-Pérez et al., 2014; Conesa et al., 2018). It is not only the dilution/dehydration effect that changes the acid concentration, but there is also an osmotic adjustment. In fact, high salinity also increases fruit acidity in citrus (Navarro et al., 2010). Under water stress, the expression levels of citrate degradation genes (*ACO* and *GAD*) are relatively low, whereas the expression levels of citrate synthase (*CS*) genes are relatively high (Zhang and Xie, 2014). However, in the BSh climate, both juice content and acids were found to be reduced and sugars increased by regulated deficit irrigation (El-Otmani et al., 2020), suggesting an interaction with temperature, as previously observed in other species (Zarrouk et al., 2016), or other environmental characteristics (for example, soil type). The relationship between high temperature and internal fruit ripening in dry climates requires further research, especially in the context of global warming, which may alter fruit quality.

Although some studies conducted in greenhouses have reported the effect of a certain indoor temperature on the internal ripening of citrus fruit (Kim et al., 2021b), the range of high temperatures and the timing that determine sugar and acid content in dry climates have not been studied under field conditions. We study the relationship between high temperature and internal fruit ripening by comparing:

1) macroclimates: fruit ripening data from 5 citrus packinghouses was studied over 6 years in two dry macroclimates, Arid climate (BSh in Taroudant located in the Souss plain, Atlantic coast of Morocco), and warm Mediterranean climate (Csa in Valencia and Castellón, Mediterranean coast of Spain).
2) microclimates: fruit ripening was studied during a whole season in 5 regions of Spain, 3 in the Mediterranean coast (two in Valencia and one in Alicante) and 2 on the Atlantic coast (Córdoba and Huelva), with a Csa climate but with different microclimatic conditions. Our analysis is based on the hypothesis that the relationship between temperature and fruit quality depends on the maximum temperature threshold and the timing and duration of heat.

## 2. Materials and methods

### 2.1. Plant material and experimental design

#### 2.1.1. First experiment. Macroclimates

The first experiment was carried out on adult 'Clementine' (*C. clementina* Hort. Ex Tanaka) mandarin orchards. The varieties selected were 'Clemenules' and 'Nour', both spontaneous mutations of 'Clementina fina', and 'Orogrande', derived from a spontaneous mutation of 'Clemenules'. The orchards were in Morocco, with an arid climate (BSh), and Spain, with a Mediterranean climate (Csa) (Peel et al., 2007). Those from Morocco were in the region of Souss Massa (Tadourant, Ouled Berhil and Sebt Gerdane), which belongs to Agadir. Of these, 80 plots had 'Clemenules', 28 plots had 'Orogrande' and 17 plots had 'Nour'. The trees were grafted on the rootstocks 'Carrizo' citrange [*Citrus sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.] and *Citrus macrophylla*. At harvest in November, fruit from all these plots was taken to three packinghouses. At each packinghouse, five boxes (20 kg) of each variety were randomly selected and 60 fruits per box were used for maturity analysis. In Spain, all 150 'Clemenules' orchards were grafted on 'Carrizo' citrange and were in the provinces of Castellón and Valencia. At harvest, fruit from all these plots was transported to two packinghouses and the same sampling procedure was used for fruit quality analyses.

#### 2.1.2. Second experiment. Microclimates

The second experiment was carried out on adult trees of sweet orange (*Citrus sinensis* L. Osb) cvs. 'Navelina' and 'M7'. The cv. 'M7' is a recent spontaneous mutation of 'Navelina' that occurred in Australia (2004) and is characterized by earlier fruit ripening, allowing earlier harvest. To obtain as much variability as possible in the internal ripening of the fruit, 5 orchards with both varieties were selected, with differences in Mediterranean (Csa) microclimate. These were in Valencia (two orchards), Alicante, Córdoba and Huelva. The rootstock used in all the farms is 'Carrizo' citrange, except in Orihuela, where the trees were grafted on *Citrus macrophylla*. Five trees of each variety, homogenous in age, vigor and good sanitary conditions were selected on each farm, and 15 fruits per tree were randomly sampled on five sampling dates between September and harvest date (November-December) and processed for maturity analyses as 5 independent replicates.

### 2.2. Soil conditions and cultural practices

The soil textures were sandy loam (Agadir, Valencia II, Alicante and Huelva), clay loam (Valencia I and Castellón) and clay (Córdoba). The pH was mostly alkaline (7.1–8), except in Huelva (6.5), and the salinity was low (0.2–0.4 dS $m^{-1}$), except in Alicante (3 dS $m^{-1}$).

Cultural practices were in accordance with normal commercial practices in each region. In both countries, the trees were irrigated with two lines of drippers per row, placed 0.75–1.0 m from the trunk for mandarin and orange trees, respectively. The drippers were placed 60–70 cm apart on the line and had a flow rate of 4 l $h^{-1}$. Water was applied once a day in the morning according to ETc. The trees were pruned in January and fertilized from March to the beginning of September with 500, 100 and 300 g $tree^{-1}$ $year^{-1}$ of N, $P_2O_5$ and $K_2O$ for the clementine mandarins and 600, 150 and 350 g $tree^{-1}$ $year^{-1}$ of N, $P_2O_5$ and $K_2O$ for the oranges. Weeds, diseases, and pests were controlled according to local criteria and regulations.

### 2.3. Climatic data

For the arid climate, the climate data were extracted from weather stations located in Taroudant (Lastah) provided by the Agrotech company of Souss Massa. For the Mediterranean climate, climatic data were obtained from the public weather stations (www.ivia.es; www.juntadeandalucia.es) closest to the plots. For the Huelva plot, data from its own weather station were used.

### 2.4. Fruit quality measurements

Color, fruit weight, juice weight, volume, acidity, and TSS were evaluated, and 3 samples of the extracted juice were stored at 4 °C until metabolomic analysis. Fruit color was determined using a color meter (Konica Minolta, CR-400). Total soluble solids (TSS; in%) were determined using a digital refractometer (Atago 3810). Juice titratable acidity (A; in%) was obtained by juice titration using 0.1 N NaOH solution.

Given the greatest differences in fruit maturity between Huelva and Córdoba, fruits from these plots were selected for metabolomic studies. The analysis of primary metabolites was carried out at the Metabolomics Service of the Institute of Molecular and Cellular Biology of Plants (IBMCP, Valencia, Spain). The analysis of sucrose, glucose, fructose, citric acid, malic acid, and succinic acid was carried out by derivatization followed by GCMS. Aliquots of 0.1 and 5 µl of fresh juice were mixed with 3 µl of internal standard (0.2 mg/mL ribitol in water) and reduced to dryness in a Speed-Vac to cover a wider range of metabolite abundance. For derivatization, the dried residues were re-dissolved in 40 µl methoxyamine dehydrochloride 20 mg/ml in pyridine and incubated at 37 °C for 90 min, followed by the addition of 60 µl MSTFA (N-methyl-N-[trimethylsilyl]trifluoroacetamide) and 6 µl of a standard retention time mixture (3. 7 % [w/v] mixture of fatty acid methyl esters between 8 and 24 °C) followed by incubation at 37 °C for 30 min. Sample volumes of 2 µl were injected in the 1/10 split mode into a 6890 N gas chromatograph (Agilent Technologies Inc. Santa Clara, CA) coupled to a Pegasus 4D TOF mass spectrometer (LECO, St Joseph, MI). Gas chromatography was performed on a BPX35 column (30 mx 0.32 mm x 0.25 m) (SGE Analytical Science Pty Ltd, Australia) with helium as carrier gas at a constant flow rate of 2 ml/min. Coating was set at 230 °C. The oven program was 85 °C for 2 min, 8 °C/min ramp to 360 °C. Mass spectra were collected at 6.25 spectra s-1 in the *m/z* range 35–900 and ionization energy 70 eV. Chromatograms and mass spectra were evaluated using the CHROMATOF program (LECO, St. Joseph, MI). For absolute quantification, peaks were compared with those of external standards and a standard curve was constructed with external standards.

### 2.5. Statistical analyses

Statgraphics CenturionXVI was used for the statistical analysis of the data. Regression analysis and analysis of variance (ANOVA) were performed, using the Kolmogórov-Smirnof test to check the normality of the data. The *arcsin* transformation was applied to the percentages. The LSD value was calculated for the mean separation study at 95 % significant level.

## 3. Results

### 3.1. First experiment. Macroclimates

The main differences between the arid (BSh) and Mediterranean (Csa) climates over the 6 years studied were the average monthly maximum temperature ($t_M$) and the average monthly minimum relative humidity (RHm). This was particularly important during summer (Fig. 1). In the arid climate, the 6-years average $t_M$ during summer (June 21st – September 21st) was significantly higher (33 °C) than in the Mediterranean climate (29 °C), and mean RHm was significantly lower (35 % and 46 %, respectively). Therefore, the differences in vapor pressure deficit (VPD) between the two climates increased during the summer compared to the spring. On the other hand, the main similarity between the BSh and Csa dry climates was the low precipitation during summer, as expected, averaging 0.2 mm and 1.1 mm respectively. Other climatic variables (minimum and average temperature, maximum and average relative humidity) showed smaller differences (data not shown). In the BSh climate, the first 4 years studied had drier summers, with a mean $t_M$ of 35 °C, a mean RHm of 29 % and a mean VPD of 1.9 kPa, compared to the last 2 years studied, which were milder and similar to the Csa summer. The mean summer data in the Csa climate did not vary significantly over the 6-year period (Fig. 1).

Under these conditions, ripe clementine mandarins showed significant differences in quality between the two climatic regions (Fig. 2). Fruit harvested in the BSh climate had significantly ($P = 0.0001$) lower juice content (43% vs 54 %), significantly ($P = 0.0001$) lower TSS (9.4 *vs* 11.1) and significantly ($P = 0.0063$) higher acidity (1.20% vs 0.95 %) than fruit harvested in the Csa climate over the 6 years of study, regardless of cultivar (Fig. 2). While fruit ripening did not vary significantly between years in the Csa climate, this was not the case in the BSh climate. In 2012, the hottest and driest summer (the lowest RHm) in Taroudant, both 'Clemenules' and 'Orogrande' fruit had 40 % juice, whereas in 2013 and 2014, with a milder summer, the fruit juice

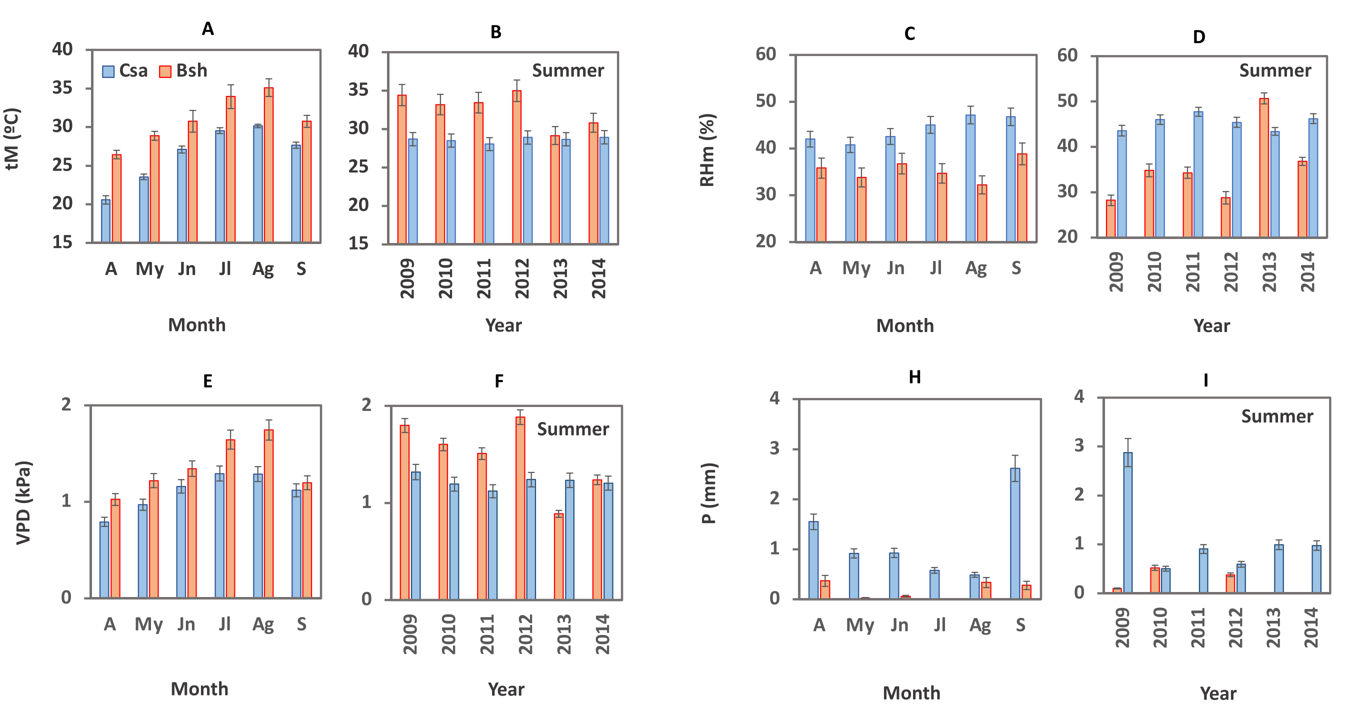


**Fig. 1.** Average monthly (A, C, E, H) or summer (June 21st – September 21st) (B, D, F, I) maximum temperature $t_M$, VPD, minimum RH (RHm) and rainfall in the Csa and BSh climates of Valencia-Castellón (Spain) and Taroudant (Morocco), respectively. Data are mean ± error standard of three weather stations.

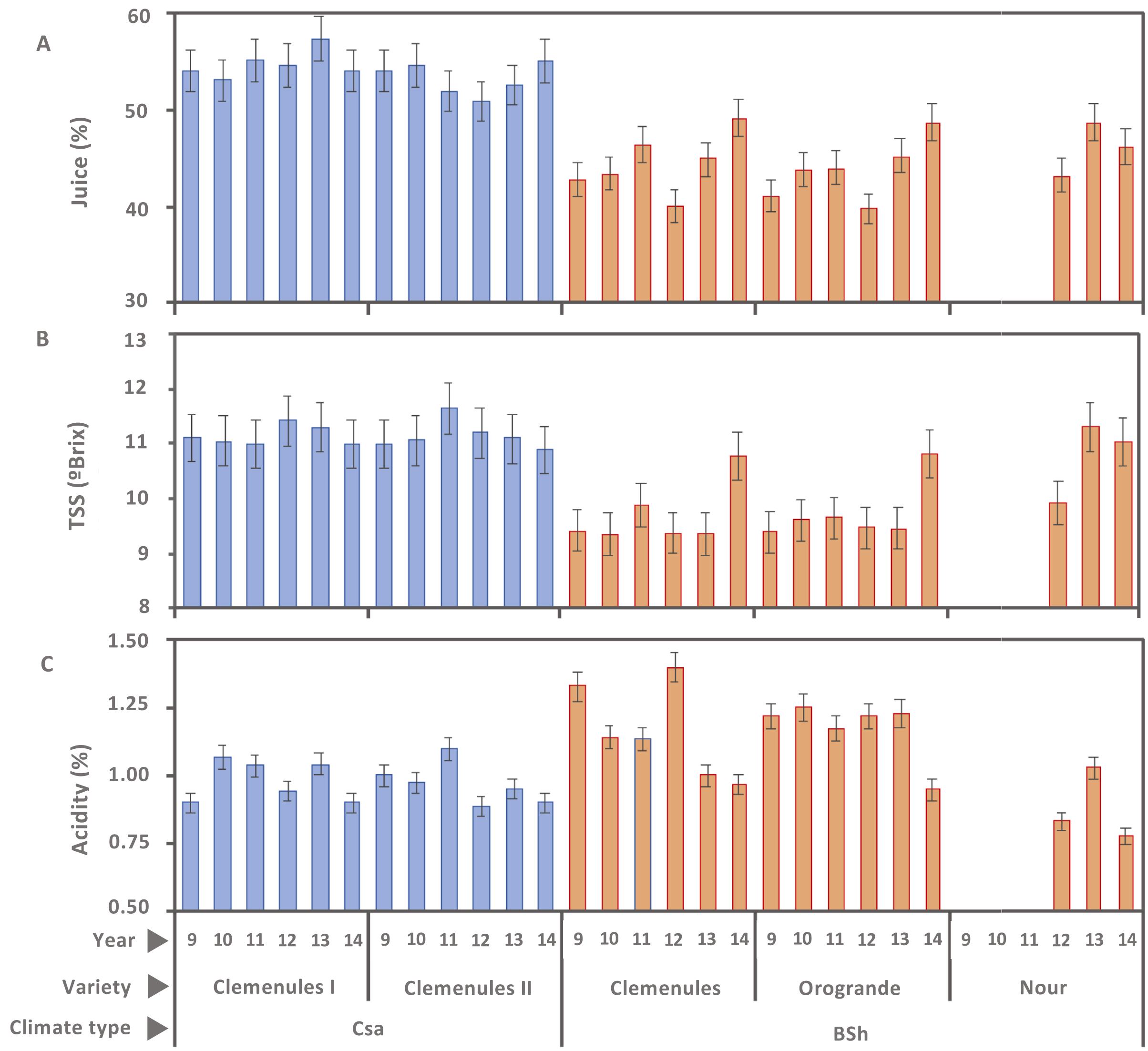


**Fig. 2.** Internal fruit quality of clementine mandarins grown in Csa (blue, Valencia and Castellón, Spain) and BSh (orange, Taroudant, Morocco) climates. The fruits were analyzed at the beginning of October (weeks 40–42 of the year for the years 2009 – 2014). Each value is the average of 300 fruits sampled in 2 packhouses in Valencia and 3 in Taroudant. Vertical bars show the standard error. Numbers 9–14 indicate years 2009–2014.

reached 46–49 %. On the contrary, 'Clemenules' fruit from Valencia and Castellón always had more than 51 % juice, with an average of 54 % (Fig. 2). The TSS content seemed to be more stable than the juice content in the BSh climate and did not change significantly in 5 out of the 6 years for both 'Clemenules' and 'Orogrande'. The highest mean TSS values were recorded in 2014 (10.8–11.2°Brix), regardless of cultivar, coinciding with mild weather conditions. Fruit acidity in the BSh climate varied significantly in 'Clemenules', it being high in hot and dry (low RHm) years (2009 and 2012) and low in mild years (2013 and 2014). In fruits from 'Orogrande' the acidity remained high in 5 of the 6 years studied, and the lowest acidity values also corresponded to the mild summer of 2014 (Fig. 2).

The behavior of the 'Nour' mandarin's deserves special attention. Although it is considered a late ripening mandarin in Morocco (Chahidi et al., 2007), it showed a lower fruit acidity compared to 'Clemenules' and 'Orogrande' (Fig. 2). This should be highlighted as an effect of the thermal change of the planet and the sensitivity of the genotype to this change. These results are important because, if the current thermal trend continues the internal ripening of the varieties may change, according to the results we have obtained by comparing different climates and regions.

The higher acidity observed during the hot and dry summers in the BSh climate suggests a climate effect on the acid synthesis phase (stage II of fruit development). Considering the latter and that $t_M$ and VPD are the main differences between the BSh and Csa climates, we studied the correlation between these two variables during the summer and fruit ripening. An increase in the number of summer days with temperatures above 30 °C correlated significantly with lower fruit TSS and juice, but not with fruit acidity (Table 1). A significant increase in the number of summer days with temperatures above 35 °C and 40 °C negatively correlates with juice content ($P = 0.0001$) and TSS ($P = 0.0001$), and positively with acidity ($P = 0.0127$). This was particularly important at the beginning of summer (July), but only in the arid climate (Table 1), because in the Csa climate the percentage of summer days above 35 °C is low (Fig. 3 and Fig. S1). Similar results were obtained with VPD, so that as VPD increased, the percentage of juice and TSS decreased ($P = 0.0001$), while acidity increased ($P = 0.0478$). These results support the hypothesis that the internal fruit quality of citrus fruits is sensitive to climatic conditions, with significant changes in arid climates ($t_M > 35$ °C and high VPD).

**Table 1**
Linear correlation (y = ax+b) between Clementine mandarin fruit quality in October (y) and the number of days reaching a given temperature (x) during summer, or during July, August, and September separately. Bold where $P < 0.01$ and $r > 0.8$. Ripening data correspond to 'Clemenules' and 'Oronules' mandarins harvested in the years 2009–2014 and 'Nour' mandarins harvested in the years 2012–2014. Climatic data correspond to Taroudant (BSh, Morocco) and Valencia-Castellón (Csa, Spain) in the same years.

| Number of days at a certain temperature | | Juice (%) | TSS (°Brix) | Acidity (%) |
|---|---|---|---|---|
| $t > 30$ °C in Csa and BSh summer | a | −0.18 | −0.04 | 0.02 |
| | r | −0.75 | −0.63 | – |
| | p-value | 0.0001 | 0.001 | n.s. |
| $t > 35$ °C in Csa and BSh summer | a | −0.31 | −0.05 | 0.008 |
| | r | **−0.82** | **−0.81** | 0.74 |
| | p-value | **0.0001** | **0.0001** | 0.019 |
| $t > 40$ °C in Csa and BSh summer | a | −0.30 | −0.06 | 0.008 |
| | r | −0.53 | −0.59 | 0.52 |
| | p-value | 0.009 | 0.003 | 0.021 |
| $T > 35$ °C in BSh July | a | −0.34 | −0.04 | 0.014 |
| | r | **−0.84** | −0.59 | 0.71 |
| | p-value | **0.0007** | 0.039 | 0.009 |
| $T > 35$ °C in BSh August | a | −0.32 | 0.04 | 0.012 |
| | r | −0.76 | −0.51 | 0.60 |
| | p-value | 0.0038 | n.s. | 0.05 |
| | a | −0.035 | 0.04 | 0.02 |
| | r | −0.04 | −0.13 | −0.63 |
| | p-value | n.s. | n.s. | n.s. |

### 3.2. Second experiment. Microclimates

The second experiment compared 5 regions of Spain, 3 in the Mediterranean and 2 in the Atlantic coast, with Csa climate but with different microclimatic conditions. To relate these conditions to citrus ripening we used sweet orange genotypes, 'Navelina' (wild type) and 'M7' (early ripening mutant). The analysis of variance shows that genotype significantly determined juice TSS content, glucose, fructose, and sucrose concentrations, whereas environment significantly determined the acid concentration, especially citric and succinic acids (Table 2). Significant differences in TSS and carbohydrates between the two genotypes and in acids between the five regions were observed in September, when the fruit was still in the immature green stage. These differences continued for sugars (between genotypes) and reduced for acids (between climates) throughout the ripening process of the fruit (Fig. 4). The acid concentration was significantly lower in the regions with hotter summers and autumns (Valencia II and Córdoba), suggesting an effect during the acid catabolism phase (end of stage II and stage III of fruit development). This observation was confirmed by the significant ($P < 0.01$) negative correlation between the number of days reaching 27 to 33 °C and fruit acidity, i.e. the higher the number of summer days reaching this temperature range, the lower the fruit acidity. The latter was particularly important for September temperatures. The slope of the regression line decreased significantly with increasing temperature within the 27 - 33 °C range (Table 3). It is important to note that the interaction between climate and variety is not statistically significant, except for succinic acid (Table 2). However, in this case it does not matter because succinic acid has little correlation and significance with total acidity (Fig. S2).

## 4. Discussion

The present study shows that in dry climates the relationship between temperature and fruit quality depends on the $t_M$ (the average monthly maximum temperature) threshold and the timing of the onset and the duration of the heat. The correlations found are statistically significant and can be accepted as reflecting valid effects, although we assume that the study does not establish a cause-effect relationship but is descriptive. Our approach is conceptually similar to that of Hutton and Landsberg (2000) conducted with 'Navel' and 'Valencia' oranges in Australia (BSk climate), but with more clear results. Whereas they proposed a temperature sum model (effective heat units above 13 °C) for the entire fruit growing season to predict relative changes in acid and sugar content at harvest time, and found a negative relationship ($R^2$ = 0.24–0.50), we propose a model in which the number of days reaching a given $t_M$ during the summer is highly correlated with fruit quality, with $R^2$ between 0.70 and 0.92 depending on the factors and variables.

The relationship between $t_M$ and acidity was stronger than that for sugar content, regardless of climate type, which corroborates previously results (Hutton and Landsberg, 2000). Thus, while TSS content was not conditioned in the Csa climate, and varied only for navel orange genotype, it was negatively correlated with heat stress in July and August in the BSh climate. On the other hand, acidity was always conditioned by temperature. In the BSh climate, heat stress above 35 °C during the acid synthesis phase (July) correlated positively with acidity, whereas high temperature (27 °C – 33 °C) during the acid reduction phase (August and September) correlated negatively with acidity in the Csa climate. This result is supported by previous studies showing that temporality of acidity reduction is influenced by environmental conditions during the second stage of fruit development (Julhia et al., 2019). While acid reduction by high temperature has been reported under controlled conditions for a given temperature (Kim et al., 2021; Reuther, 1973; Sadka et al., 2019), we report the range of temperatures under field conditions that are highly correlated (P-value between 0.0004 and 0.009; r between −0.81 and −0.92) with reduced fruit acidity. The reduction of fruit acidity due to high temperature was explained by Etienne et al. (2013) through two pathways: 1) an increase in respiration and the activity of enzymes involved in the tricarboxylic acid (TCA) cycle and glycolysis which reduce the citrate concentration, and 2) a reduction of the vacuolar storage of citrate due to changes in the tonoplast permeability and the activity of proton pumps.

A novel finding of our study is the positive relationship between heat stress and acidity in the arid-dry climate, which has never been reported. However, our results cannot confirm whether the higher acidity is a direct effect of temperature or indirect through water status. The fact that heat stress correlated negatively with juice and TSS content, but positively with acidity, suggests a mechanism involving metabolic effects, in addition to the simple dehydration effect. Although the trees were irrigated at 100 % of the estimated ETc, our study cannot exclude the water status of the plant, considering the sandy soil texture in our experiment and the well-known effect of summer RDI on increasing fruit acidity (Ballester et al., 2011, 2013, 2014). The extreme summer temperatures in arid conditions and the higher VPD (2012 higher than 2013) favor water stress and, as an osmotic compound, citric acid plays an important role in the response to water stress. In fact, induced water stress (−40 % irrigation) on 'Ponkan' mandarin reduces the expression levels of citrate degradation genes (*ACO* and *GAD*) and increases the expression levels of citrate synthesis genes (*CS*) (Zhang and Xie, 2014) which may explain the accumulation of citric acid during water stress. The fact that the total amount of juice per fruit was always lower in the arid climate than in the Mediterranean climate supports the water stress effect. Fruit size was not reduced due to a higher peel growth in the arid conditions (data not shown). In the Tropical climate of Florida, similar results were found regarding the relationship between water withholding from the root zone of citrus trees during stage II of fruit development and juice osmotic adjustment (Barry et al., 2004).

The extreme summer temperatures in arid conditions and the higher VPD significantly reduce net photosynthesis (when $t > 35$ °C), stomatal conductance and transpiration (El-Sharkawy et al., 1985; Guo et al., 2006), which would explain the lower transport of sugars to the fruit and

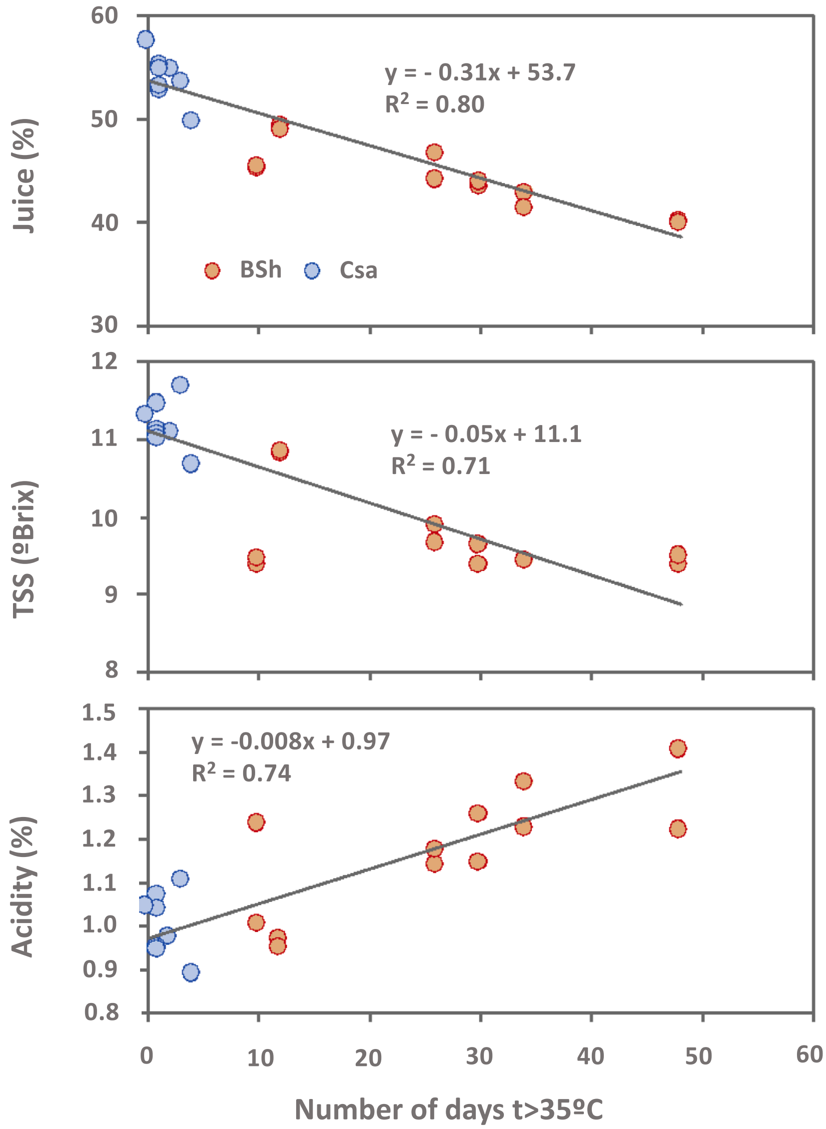


**Fig. 3.** Linear correlation (y = ax+b) between Clementine mandarin fruit quality parameters in October (y) and the number of days with temperature greater than 35 °C during summer in the Mediterranean (blue) and Arid (orange) climates. The equation and trendline include all the points.

**Table 2**
Effect (P-value) of environment (Valencia I, Valencia II, Alicante, Córdoba, and Huelva) and genotype ('M7' and 'Navelina') on sweet orange fruit acidity (%), concentration of citrate, succinate and malate, TSS (°Brix) and concentration of sucrose, glucose and fructose. Fruit was sampled in September. Bold where $P < 0.01$.

| | Environment ($n = 5$) | Genotype ($n = 2$) | E x G |
|---|---|---|---|
| Acidity | **0.0001** | 0.2716 | 0.3022 |
| Citrate | **0.0001** | 0.3618 | 0.3158 |
| Succinate | 0.0294 | **0.0050** | 0.0130 |
| Malate | 0.1947 | 0.4103 | 0.4403 |
| TSS | 0.1460 | **0.0001** | 0.3966 |
| Sucrose | 0.1684 | **0.0012** | 0.3328 |
| Glucose | 0.1299 | **0.0003** | 0.5325 |
| Fructose | 0.3486 | **0.0014** | 0.3977 |

the negative correlation with TSS. The fact that there was a delay in the accumulation of TSS in 2012 (more extreme) compared to 2013 supports this hypothesis. Indeed, the TSS content in week 36 in 2012 shows an average value of 8.2°Brix, while in 2013 it is 9.3°Brix, but the values are equalized in week 41 with values of 9.7°Brix, when the temperature decreased.

In conclusion, heat stress in dry climates correlates with changes in fruit quality, juice, acids and sugars, depending on the $t_M$ threshold and the timing of the onset and duration of heat. In the arid climate (BSh), the heat stress threshold was established at a significant number of days above 35 °C. This was particularly important at the beginning of summer (July in the Northern Hemisphere). Currently, in the Mediterranean climate (Csa), the percentage of summer days above 35 °C is still low (Fig. S1) and the temperature between 27 °C – 33 °C only affects the reduction of fruit acidity. However, the percentage of days during the summer above 30 °C has increased from 30 % to 80 % in the last 20 years (Fig. S1) and, according to the World Meteorological Organization (www.wmo.int, 2023), 2023 has been the hottest year on record. The Mediterranean countries were particularly hit by extreme heat, especially in the second half of July, when Italy recorded 48.2 °C, Tunis (Tunisia) 49.0 °C, Agadir (Morocco) 50.4 °C and Algiers (Algeria) 49.2 °C. Our results will therefore help to interpret future changes in citrus

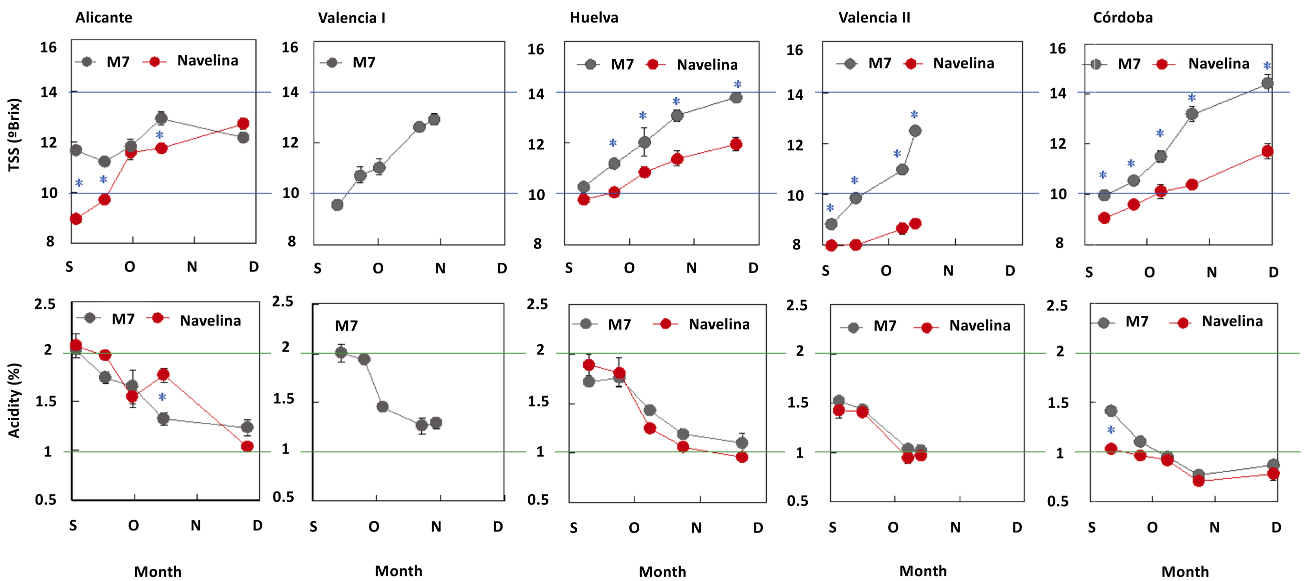


**Fig. 4.** Time-course of TSS (°Brix) and acidity (%) of 'M7' and 'Navelina' sweet orange in 5 regions in Spain with Mediterranean climate. * Indicates significant differences ($P < 0.05$). The letters S, O, N, D, are for the months of September, October, November, and December. The last date is the commercial harvest of the early ripening M7 cultivar.

**Table 3**
Linear correlation between orange fruit acidity in September (before harvest) and the number of days reaching a given temperature during summer, August, and September. Bold where $P < 0.01$ and $r > 0.8$. Data correspond to five regions (Valencia I, Valencia II, Alicante, Córdoba, and Huelva) and two genotypes (M7 and Navelina).

| | Summer (21Jn-21Sp) | | | August | | September | |
|---|---|---|---|---|---|---|---|
| Temperature (°C) | P-value | a | r | P-value | r | P-value | r |
| 25 | n.s. | n.s. | n.s. | 0.1891 | −0.48 | 0.0100 | −0.79 |
| 26 | 0.0253 | −0.11 | −0.73 | 0.1957 | −0.47 | 0.0111 | −0.79 |
| 27 | **0.0003** | **−0.09** | **−0.93** | 0.1967 | −0.47 | **0.0087** | **−0.81** |
| 28 | **0.0001** | **−0.04** | **−0.95** | 0.0626 | −0.81 | **0.0031** | **−0.86** |
| 29 | **0.0002** | **−0.04** | **−0.94** | 0.0627 | −0.64 | **0.0061** | **−0.82** |
| 30 | **0.0001** | **−0.03** | **−0.96** | **0.0013** | **−0.89** | **0.0012** | **−0.89** |
| 31 | **0.0001** | **−0.03** | **−0.95** | 0.0127 | −0.78 | **0.0004** | **−0.92** |
| 32 | **0.0009** | **−0.02** | **−0.90** | 0.037 | −0.70 | **0.0014** | **−0.89** |
| 33 | **0.0021** | **−0.02** | **−0.87** | 0.0203 | −0.75 | **0.0037** | **−0.85** |
| 34 | 0.0131 | −0.01 | −0.78 | 0.0530 | −0.66 | 0.0120 | −0.84 |
| 35 | 0.0132 | −0.01 | −0.79 | 0.0740 | −0.70 | 0.0150 | −0.84 |

fruit quality due to global warming. However, further research is needed to confirm the direct effect of temperature on internal ripening of citrus fruit. It would also be useful to find the temperature-hour exposure threshold that determines fruit internal ripening, as it was established for fruit external ripening (Mesejo et al., 2012).

## CRediT authorship contribution statement

**Carlos Mesejo:** Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **Amparo Martínez-Fuentes:** Writing – review & editing, Methodology, Investigation, Data curation. **Carmina Reig:** Writing – review & editing, Methodology, Investigation, Data curation. **Mohamed El-Otmani:** Writing – review & editing, Conceptualization. **Manuel Agustí:** Writing – review & editing, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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During the preparation of this work the authors used DeepLWrite to improve language and readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2024.113501.

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