Storage Temperature Effects on Blood Orange Fruit Quality

Paolo Rapisarda,* Santina Elisabetta Bellomo, and Sebastiano Intelisano

Istituto Sperimentale per l'Agrumicoltura, Corso Savoia 190, 95024 Acireale, Italy

Orange fruits of two blood varieties (Tarocco and Moro) were stored at 8 °C and 22 °C for 85 and 106 days, respectively, and analyzed periodically for standard quality parameters (total soluble solids, total acidity, ascorbic acid, juice yield, and rind color) and sensory influencing parameters (anthocyanins, and total and free hydroxycinnamic acids). A decrease in total acidity (TA) and juice yield during storage was observed for both cultivars; total soluble solids (TSS) increased only in the Tarocco oranges stored at 8 °C. The increase in TSS observed for Tarocco and the simultaneous decrease in TA in both varieties resulted in a higher maturity index (TSS/TA) for the two cultivars. No loss of vitamin C was noted in Tarocco orange at either temperature, whereas a sharp reduction in vitamin C occurred in the first 50 days of storage for Moro. A significant increase in anthocyanin content was observed in Tarocco and Moro stored at 8 °C. Overlong storage induces extensive hydrolysis of hydroxycinnamic derivatives to free acids in Moro orange and these, in turn, could develop the malodorous vinylphenols.

Keywords: *Pigmented orange; postharvest; anthocyanins; hydroxycinnamic acids*

INTRODUCTION

In Italy approximately 70% of sweet orange [C*itrus sinensis* (L.) Osbeck] production is represented by the blood varieties Tarocco, Moro, and Sanguinello (*1*). These cultivars are characterized by their unique flesh and rind color due to red pigments belonging to the anthocyanin class (*2*, *3*). Another peculiar characteristic of blood oranges is the higher concentration of hydroxycinnamic acids and flavanones than in blond orange varieties (*4*, *5*). As well as affecting fruit-sensory influencing qualities, these substances have an important biological role owing to their antioxidant activity (*6*, *7*).

In the postharvest phase, orange fruits can be stored at low temperatures for more or less extended periods (*8*). Despite the fact that oranges are considered nonclimacteric fruits, such treatments can give rise to increased respiration because of possible chilling injury (*9*) as well as biochemical changes in the fruit. In addition, blood orange varieties present problems during storage because of their high susceptibility to chilling injury when stored below 8 °C (*10*), and to altered fragrance caused by the onset of unpleasant off-flavors.

Recent studies have shown that storage temperature affects the sensory-influencing qualities of blood orange juice. In particular, vinylphenol concentrations (the malodorous substances that arise from free hydroxycinnamic acid decarboxylation) in juices stored at 4 °C and at 25 °C for over four months, exceeded the odor threshold value (*11*).

The objective of this work was to study the effects of two storage temperatures, 8 and 22 °C, on standard and sensory-influencing quality parameters of Tarocco and Moro orange fruits in order to contribute to the knowledge of metabolic processes that occur during postharvest treatment of the fruits.

* To whom correspondence should be adressed. Fax: 0039-95-765-3113. E-mail: isa@mail.gte.it.

MATERIALS AND METHODS

The study was carried out on fruits of Tarocco 'Galici' and Moro 'OL 8D' cultivars, harvested in February (Tarocco) and March (Moro) 1999 at commercial maturity (minimum TSS/TA ratio of 7.0 for Moro and 8.0 for Tarocco oranges, according to Italian law). The fruits were harvested from trees approximately 20 years old grown at the Palazzelli experimental farm of the Istituto Sperimentale per l'Agrumicoltura (Acireale, Italy) in the territory of Lentini (Siracusa, Italy). Once in the laboratory, fruits of both varieties underwent fungicide treatment with a solution containing 1000 mg/L of Imazalil. Fruits were then kept dry at room temperature for ca. 6 h before being placed randomly in boxes (50 fruits per box). For each of two temperature treatments and for each of the two cultivars, 36 boxes were used (12 samples in triplicate form) and stored in two temperature-controlled chambers held, respectively, at 8° ± 2 and 22° ± 2 with a relative humidity (RH) of 90−95% and with complete air change every hour. Sampling of the fruits (twenty per box) for physicochemical analyses was performed before storage (time 0) and ca. every 10 days for a total storage period of 85 days for the Tarocco and 106 days for Moro fruits.

Color Determination. Rind color was measured along the equatorial axis of whole fruit using a Minolta Chromameter CR-200 (Minolta Camera Co. Ltd., Osaka, Japan) to determine CIELAB parameter a* (redness), b* (yellowness), and a*/b* ratio.

Analytical Methods. Juice extracted with a domestic juicer was sampled for total acidity (TA), total soluble solid (TSS), ratio (TSS/TA), vitamin C, anthocyanins, and free and total hydroxycinnamic acids.

TSS and TA (as anhydrous citric acid) were determined according to standard methods (*12*). Vitamin C was determined by the 2,6-dichlorophenolindophenol titrimetric method modified by Rapisarda et al. (*13*). Anthocyanins were analyzed spectrophotometrically by the pH differential method (*14*, *15*) and expressed as cyanidin-3-glucoside concentration.

Extraction and Determination of Hydroxycinnamic Acids. Free hydroxycinnamic acids (caffeic, sinapic, *p*-coumaric, and ferulic) were extracted from juice using solid-phase extraction (SPE) of samples on a C18 cartridge. An aliquot of centrifuged juice (5 mL) was acidified with 1 N HCl to pH 2.5 and then passed through a Varian Mega Bond Elut C18

10.1021/jf010032l CCC: $20.00
Published on Web 06/28/2001

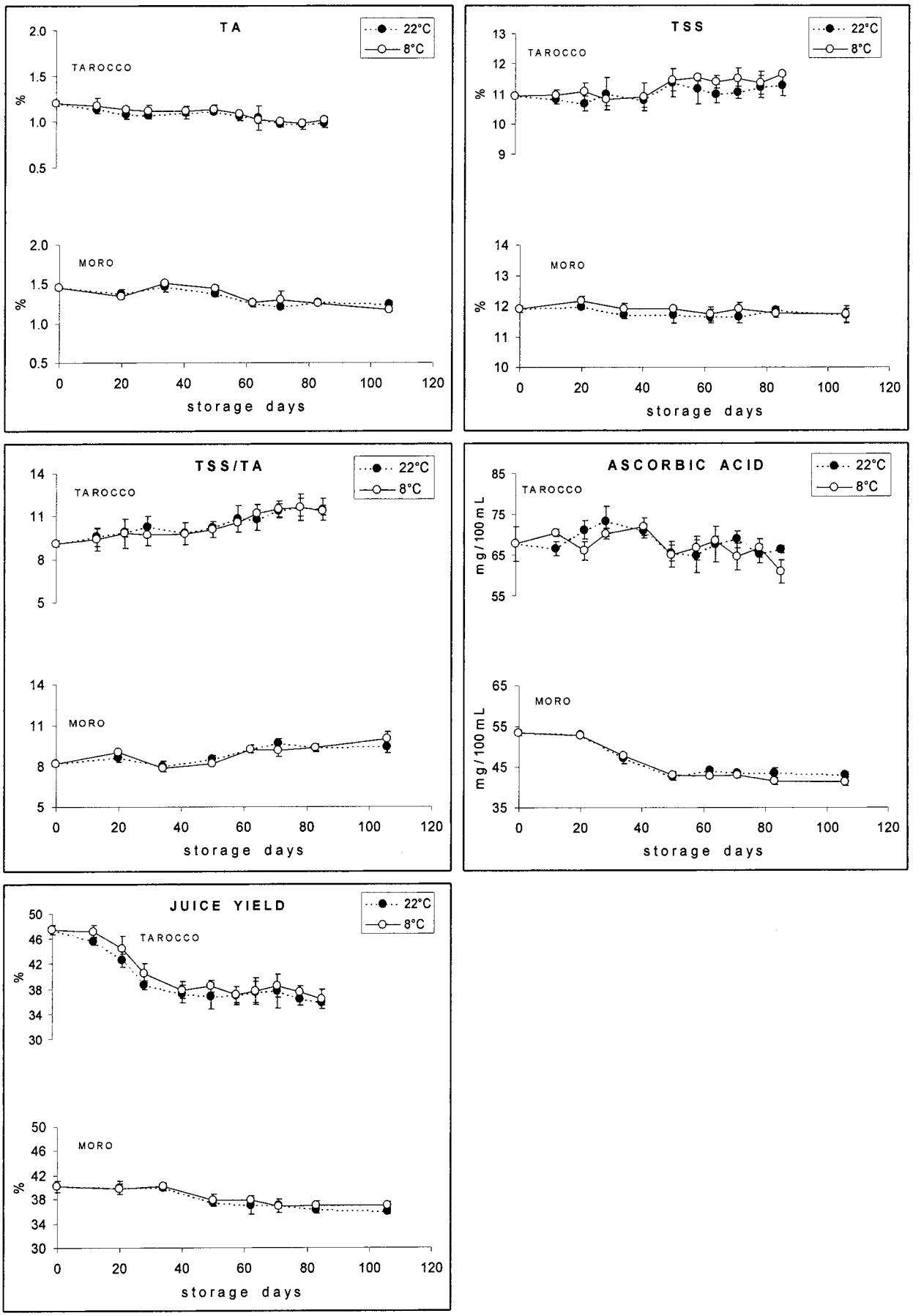

Figure 1. Change in TA, TSS, TSS/TA, ascorbic acid, and juice yield in Tarocco and Moro fruits during storage at 8 °C and 22 °C.

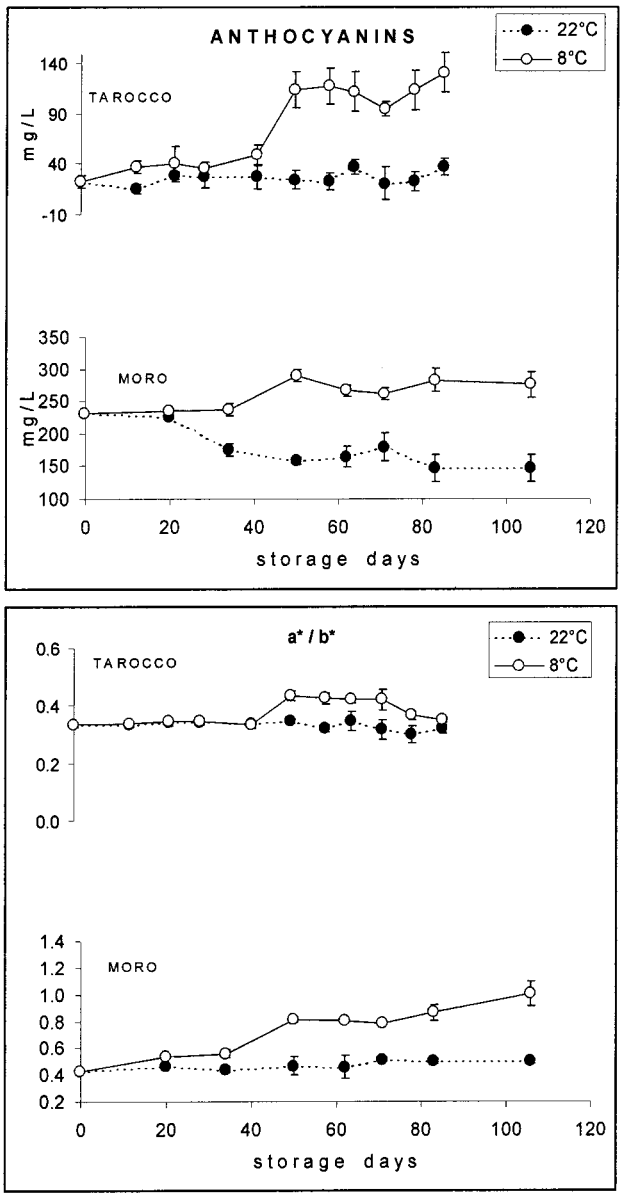

Figure 2. Change in anthocyanin content in juices and color parameters ratio of Tarocco and Moro fruits stored at 8 °C and 22 °C.

cartridge previously conditioned with acidulated water (0.01 N HCl). The cartridge was rinsed with water and then eluted with 1% HCl methanol. The alcoholic solution was vacuum evaporated, and the residue was diluted with 5 mL of HPLC mobile phase (see later).

Total hydroxycinnamic acids were extracted after alkaline hydrolysis of hydroxycinnamic esters. In this case 10 mL of centrifuged juice was added to 10 mL of 2 N NaOH and stored at room temperature in the dark. Complete hydrolysis of bound forms of hydroxycinnamic acids occurred in 4 h. The solution was then acidified with 2 N HCl to pH 2.5, and the hydroxycinnamic acids were extracted by SPE method (*4*).

HPLC Equipment and Conditions. The HPLC system consisted of a Waters model 600E solvent pump equipped with a W-484 UV−Vis detector, a W-746 Data Module Integrator (Waters Associated, Milford, MA), a Rheodyne injection valve with 20 μL sample loop and a 25 cm × 4.6 mm i.d. Hypersil ODS 5μm column (Policonsult Scientifica, Milan, Italy). The column temperature was maintained at 30 ± 2 °C and the chromatograms were recorded with a 300 nm UV light. Hydroxycinnamic acids were eluted at 1 mL/min with 2% acetic acid in water as solvent A and methanol as solvent B. A linear gradient from 5% to 30% of B was carried out in 30 min. The peak assignments were based on the retention times of the standard acids (caffeic, sinapic, *p*-coumaric, and ferulic acids, Fluka Chimica, Milan, Italy) and the chromatograms in the mixture. A calibration line of peak area versus known concentrations of standard hydroxycinnamic acids was plotted. The concentration of each acid was calculated from the experimental peak area by analytical interpolation in the standard calibration lines.

Because of the moisture loss of the fruits of the two varieties during storage (see later) all the concentrations of the single components analyzed were calculated taking into account the percentage of water lost at each stage of storage.

RESULTS AND DISCUSSION

Figure 1 shows the results obtained after determining the TA, TSS, TSS/TA, ascorbic acid content and juice yield of Tarocco and Moro orange fruits during storage at 8 and 22 °C.

Total acidity of fruits of the two cultivars decreased during storage in the same mode when stored at both 8 and 22 °C (Figure 1). Citric acid has been reported to decrease in stored citrus fruits (*8*), and this decline may be in part due to the use of organic acids for energy production and alcoholic fermentation (*16*).

A slight increase in TSS occurred only in Tarocco fruits stored at 8 °C; the TSS level in Moro fruits remained unchanged at both temperatures. Juice yield in Tarocco orange stored at two temperatures decreased from 47.5% to ca. 38% during the first 40 days, then then remained unchanged up to the end of the 85 days. Variation in juice yield for Moro was more limited (from 40 to 37%) (Figure 1).

The increase in TSS for Tarocco and the simultaneous decrease in TA in both varieties resulted in an increase of maturity index (TSS/TA) which rose from 9.1 to 11.5 for Tarocco and from 8.3 to 10.0 for Moro (Figure 1). Increase of the TSS/TA ratio during storage has been observed in the blood orange Tarocco (*17*, *18*), in Hamlin and Valencia orange (*19*, *20*) and in grapefruit (*21*). A high TSS/TA ratio has generally been regarded as a quality index for orange fruits, but its increase during storage can also be accompanied by the development of off-flavors due to formation of ethanol in the fruit (*22*).

No changes in ascorbic acid content were detected in Tarocco during the storage period; but levels in Moro diminished sharply, particularly in the first 50 days, then stabilized for the remaining storage period. The evolution of this parameter over time for the two cultivars was not affected by the storage temperature values in both cases. The stability of vitamin C in fruit during storage has been extensively studied because citrus fruits and their products are one of the largest suppliers of dietary vitamin C (*23*). Various reports have shown that vitamin C loss in citrus fruits during storage at different temperatures is slight (*23*). In the present case the initial enhanced decay of ascorbic acid in Moro could be ascribed to an interaction with anthocyanins which are present in high concentrations in fruits (Figure 2). In fact, direct condensation with anthocyanins may account for this decrease (*24*, *25*). The much lower pigment level in Tarocco orange accounts for the negligible degradation of ascorbic acid. Previous studies of Tarocco orange have also shown that no loss in vitamin C occurs during storage of the fruits at low temperatures (*17*, *18*).

Blood orange principally contains cyanidin-3-glucoside and cyanidin-3-(6″-malonyl)-glucoside (*26*) and such pigments may also undergo changes during post-harvest

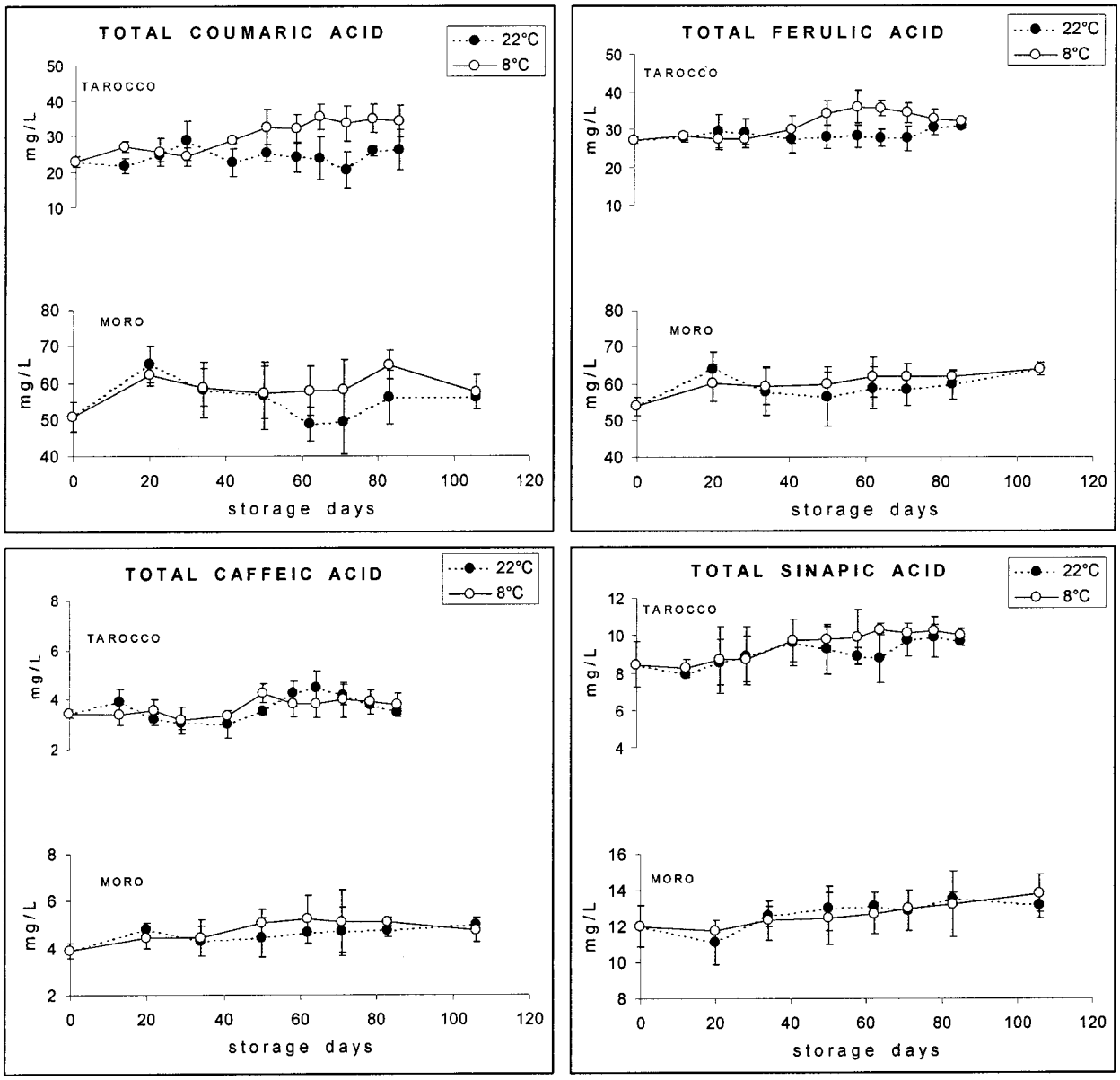

Figure 3. Change in concentration of total hydroxycinnamic acids in Tarocco and Moro fruits stored at 8 °C and 22 °C.

treatments. During storage of Tarocco fruits at 8 °C, anthocyanin concentrations rose by about 500% from an initial 22 mg/L to 130 mg/L, whereas values for Moro increased by only about 19% (from 231 to 276 mg/L) (Figure 2). In both varieties stored at 8 °C, the highest production of anthocyanins occurred after 40 days storage. In fruits stored at 22 °C pigment concentrations did not vary for Tarocco but they decreased in Moro orange. The increased pigmentation of fruits can be attributable to the activation of the enzymes involved in phenylpropanoid metabolism induced by the low-temperature according to other reports which found that cold storage stimulates anthocyanin biosynthesis in apple (*27*), pomegranate (*28*), and lowbush blueberry fruits (*29*). On the other hand, high temperatures inhibit anthocyanin formation in 'Tokai' and reduce it in 'Cardinale' and 'Pinot nero' grape varieties (*30*).

Anthocyanin accumulation on the rind of the Tarocco and Moro orange fruits stored at 8 °C was also confirmed by the increased a*/b* ratio (Figure 2). This value remained unchanged for fruits of both cultivars stored at 22 °C. It is interesting to note that the trend of the anthocyanin contents in the fruit juice (Y) during storage corresponds to that of the a*/b* values in the rind. Linear regression between the two parameters found for Tarocco stored at 8 °C yielded the following equation: $Y = -183.3 + 690.42X$ with $r = 0.696$*** ($p < 0.001$). Establishment of this relationship between anthocyanin content in Tarocco fruit juice and a*/b* ratio values makes the recognition of internal pigmentation of fruits possible with a nondestructive method.

Citrus fruits contain esters and glycosides of hydroxycinnamic acids (*31*). Recent studies have shown that hydroxycinnamic acids were more abundant in blood orange than in blond orange fruit juices and ferulic acid was predominant over the other hydroxycinnamic acids (*4*). Ferulic and *p*-coumaric acids were also precursors of *p*-vinylguaiacol and *p*-vinylphenol, respectively, which are two detrimental off-flavors that form in blood orange juice during storage (*11*). Thus, the variations of concentrations of free and bound hydroxycinnamic acids during storage might provide a reliable index of blood orange fruit quality.

Figure 3 shows the variations in the total hydroxycinnamic acid concentrations in the stored fruits. In Tarocco fruits stored at 8 °C *p*-coumaric and ferulic acid

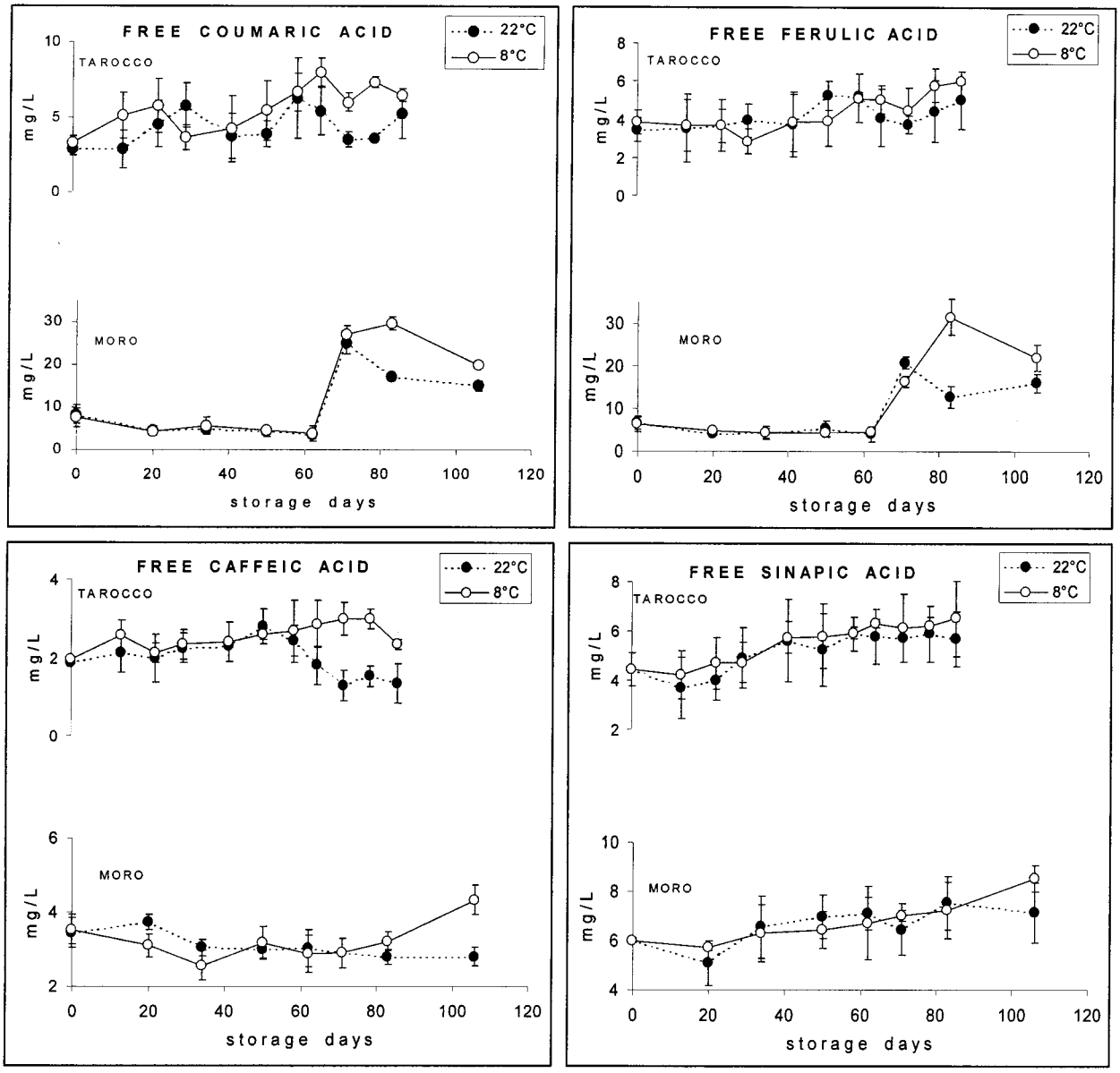

Figure 4. Change in concentration of free hydroxycinnamic acids in Tarocco and Moro fruits stored at 8 °C and 22 °C.

levels increased after the thirtieth day of storage, whereas no significant variation in these acid values was observed for fruits stored at 22 °C. Increases in caffeic and sinapic acid contents were more limited at both temperatures. The same trend was also noted in the Moro cultivar. This confirms the positive effect of low temperatures on phenylpropanoid metabolism. In fact, the first step of this pathway is the conversion of L-phenyalanine to *trans*-cinnamic acid catalyzed by phenylalanine ammonia-lyase (PAL). Successive hydroxylation and/or methylation of *p*-coumarate yields caffeic, ferulic, and sinapic acids (*32*).

The concentrations of free coumaric, *p*-ferulic, and sinapic acids in Tarocco orange did not vary at 22 °C, whereas a sharp reduction in free caffeic acid content was observed after 50 days storage at this temperature. A slight increase in free, *p*-coumaric, ferulic, caffeic, and sinapic acids occurred at 8 °C (Figure 4).

In the Moro orange, a dramatic increase of the free coumaric and *p*-ferulic acids was noted after 60 days of storage at both temperatures (Figure 4), indicating that most of the hydroxycinnamic derivatives are hydrolyzed to free acids during the last part of the period of storage. High levels of free ferulic and *p*-coumaric acids may represent the first stage leading successively to the formation of the detrimental *p*-vinylguaicol and *p*-vinylphenol by loss of CO_2, respectively, inducing an unpleasant off-flavor in the Moro fruits.

CONCLUSION

The results of this study indicated that Tarocco and Moro internal standard parameters such as TA, TSS, and TSS/TA vary slightly during storage at 8 and 22 °C and a similar trend was observed in both cultivars. Only in cv. Tarocco was a significant decrease in juice yield noted. The variation of ascorbic acid and anthocyanins was different in the two studied cultivars. The anthocyanin content suddenly increases after day 40 in Tarocco orange stored at 8 °C, but only a slight increase was observed in the Moro fruits stored at the same temperature.

Because anthocyanin levels in blood orange represent an important quality index for fresh and processed

products, an increase in pigmentation in fruits during storage at low temperatures could be commercially exploited by marketers. Besides, anthocyanin content appears to be one of the most important factors influencing the antioxidant activity of blood orange juice (*7*).

Simultaneous degradation of anthocyanins and ascorbic acid in Moro orange stored at 22 °C may occur through a direct interaction between them as evidenced by experiments carried out on model systems (*24*, *25*). Moreover, in the two cultivars studied, a balance of opposite processes could be implicated, i.e. formation and degradation of anthocyanins, the former prevailing at the low temperature and the latter prevailing at the higher one.

Finally, an overlong storage period of Moro fruits induces extensive hydrolysis of hydroxycinnamic derivatives to free acids, and these, in turn, could develop the malodorous vinylphenols, which are an index of too-advanced senescence of blood orange fruits.

ACKNOWLEDGMENT

We thank Prof. E. Maccarone of the University of Catania for his critical reading of the manuscript and A. Giuffrida for technical assistance.

LITERATURE CITED

(1) Rapisarda, P.; Giuffrida A. Anthocyanin level in Italian blood oranges. *Proc. Int. Citriculture* **1992**, 1130−1133.
(2) Maccarone. E.; Maccarrone, A.; Perrini, G.; Rapisarda, P. Anthocyanins of the Moro orange juice. *Ann. Chim.* **1983**, *73*, 533−539.
(3) Maccarone, E.; Maccarrone, A.; Rapisarda, P. Acylated anthocyanins from oranges. *Ann. Chim.* **1985**, *75*, 79−86.
(4) Rapisarda, P.; Carollo, G.; Fallico, B.; Tomaselli, F.; Maccarone, E. Hydroxycinnamic acids as markers of Italian blood orange juices. *J. Agric. Food Chem.* **1998**, *46*, 464−470.
(5) Postorino, E.; Gionfriddo, F. I flavonoidi glucosidi dei succhi di arancia italiani. *Essenze Deriv. Agrum.* **1999**, *69*, 149−158.
(6) Bonina, F.; Saija, A.; Tomaino, A.; Lo Cascio, R.; Rapisarda, P.; Dederen, C. J. *In vitro* antioxidant activity and *in vivo* photoprotective effect of a red orange extract. *Int. J. Cosmet. Sci.* **1998**, *20*, 331−342.
(7) Rapisarda P.; Tomaino, A.; Lo Cascio, R.; Bonina, F.; De Pasquale, A.; Saija, A. Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. *J. Agric. Food Chem.* **1999**, *11*, 4718−4723.
(8) Grierson, W.; Ben-Yehoshua, S. Storage of citrus fruits. In *Fresh Citrus Fruits;* Wardowski, V. F., Nagy, S., Grierson, W., Eds.; Avi Publishing Co. Inc.: Westport, CT, 1986; pp 479−507.
(9) Biale, J. B.; Young, R. E. Respiration and ripening in fruits. Retrospect and prospect. In *Recent Advances in The Biochemistry of Fruits and Vegetables*; Friend, J., Rhodes, M. J. C., Eds. Academic Press: New York, 1981; pp 1−39.
(10) Pratella, G.; Tonini, G.; Cessari, A. Postharvest disease problems of Italian citrus fruit. *Proc. Int. Citrus Symp., 1st* **1969**, *3*, 1317−1323.
(11) Fallico, B.; Lanza, M.; Maccarone, E.; Nicolosi Asmundo, C.; Rapisarda, P. Role of hydroxycinnamic acids and vinylphenols in the flavor alteration of blood orange juices. *J. Agric Food Chem.* **1996**, *44*, 2654−2657.
(12) Metodi Ufficiali di Analisi per le Conserve Vegetali − Parte Generale. MAF, D. M. 3 Febbraio 1989.
(13) Rapisarda, P.; Intelisano, S. Sample preparation for vitamin C analysis of pigmented orange juices. *Ital. J. Food Sci.* **1996**, 251−256.
(14) Rapisarda P.; Fallico, B.; Izzo R.; Maccarone E. A simple and reliable method for determining anthocyanins in blood orange juices. *Agrochimica* **1994**, *38*, 157−164.
(15) Rapisarda, P.; Fanella, F.; Maccarone, E. Reliability of analytical methods for determining anthocyanins in blood orange juices. *J. Agric. Food Chem.* **2000**, 2249−2252.
(16) Echeverria, E.; Valich; J. Enzymes of sugar and acid metabolism in stored Valencia oranges. *J. Am. Soc. Hortic. Sci.* **1989**, *114*, 445−449.
(17) Testoni, A.; Eccher Zerbini, P.; Intrigliolo, F.; Lanza, G. Influenza della concimazione azotata sulla qualità dell'arancio Tarocco vecchia linea e Tarocco nucellare durante la conservazione frigorifera. In *Il Recente Contributo della Ricerca allo Sviluppo dell'Agrumicoltura Italiana*; Delfino, C., Ed.; Sassari, 1987; pp 419−428.
(18) Schirra, M.; Chessa, I. Physiological behaviour of Tarocco oranges during cold storage. *Proceedings of the 6th International Citrus Congress* **1988**, 1491−1498.
(19) Echeverria, E.; Ismail, M. Changes in sugars and acids of citrus fruits during storage. *Proc. Fla. State Hortic. Soc.* **1987**, *100*, 50−52.
(20) Khalifah, R. A.; Knyendall, J. R. Effect of maturity storage quality of Valencia oranges. *Proc. Am. Soc. Hortic. Sci.* **1965**, *86*, 288−296.
(21) Bruemmer, H. J.; Roe, B. Post-harvest treatment of citrus fruit to increase Brix/Acid ratio. *Proc. Fla. State Hortic. Soc.* **1969**, *82*, 212−215.
(22) Davis, P.; Hoffman, R. C.; Hatton, T. T. Temperature and duration of storage on ethanol content of citrus fruits. *HortScience* **1974**, *9*, 376−377.
(23) Nagy, S. Vitamin C contents of citrus fruit and their products: A review. *J. Agric. Food Chem.* **1980**, *187*, 530−534.
(24) Poei-Langston, M. S.; Wrolstad, R. E. Color degradation in an ascorbic acid−anthocyanin−flavanol model system. *J. Food Sci.* **1981**, *46*, 1218−1222.
(25) Maccarone, E.; Passerini, A. Stabilità di antocianine in sistemi modello. *Chim. Ind.* **1990**, *72*, 890−898.
(26) Maccarone, E.; Rapisarda, P.; Fanella, F.; Arena, E.; Mondello, L. Cyanidin-3-(6"-malonyl)-β-glucoside. One of the major anthocyanins in blood orange juice. *Ital. J. Food Sci.* **1998**, *4*, 10, 367−372.
(27) Tan, S. C. Relationships and interactions between phenylalanine ammonia−lyase, phenylalanine ammonia−lyase inactivating system and anthocyanin in apples. *J. Am. Soc. Hortic. Sci.* **1979**, *104*, 581−586.
(28) Gil, M. I.; Garcia-Vignera, F.; Artes, F.; Tomas-Barberan, F. A. Change in pomegranate juice pigmentation during ripening. *J. Sci. Food Agr.* **1995**, *68*, 77−81.
(29) Kalt, W.; McDonald, J. E. Chemical composition of lowbush blueberry cultivars. *J. Am. Soc. Hortic. Sci.* **1996**, *121*, 142−146.
(30) Kliwer, M. W. Effect of day temperature and light intensity on coloration of *Vitis Vinifera* L. grapes. *J. Am. Soc. Hortic. Sci.* **1970**, *95*, 693−697.
(31) Risch, B.; Hermann, K. Hydroxycinnamic acid derivatives in citrus fruits. *Z. Lebensm. Unters. Forsch.* **1988**, *187*, 530−534.
(32) Heller, W.; Forkman, G. Biosynthesis. In *The Flavonoids Advanced in Research*; Harborne, J. B., Ed.; Chapman and Hall: London, 1988; pp 399−425.

Received for review January 4, 2001. Revised manuscript received April 30, 2001. Accepted May 7, 2001.

JF010032L