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Antioxidant capacity, ascorbic acid, total phenols and carotenoids changes during harvest and after storage of Hayward kiwifruit

Silvia Tavarini [a], Elena Degl'Innocenti [a], Damiano Remorini [b], Rossano Massai [b], Lucia Guidi [a,*]

[a] Dipartimento di Chimica e Biotecnologie Agrarie (DCBA), University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy
[b] Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi" (DCDSL), University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy

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Abstract

The influences of harvest time and storage on the quality indices and nutritional content of kiwifruit were evaluated. Antioxidant capacity, ascorbic acid, total phenol content, carotenoids, soluble solids content and flesh firmness were determined in kiwifruit gathered at two different time (T1: 17-11-2005 and T2: 24-11-2005) and stored at 0 °C, for 2 or 6 months (S1 and S2, respectively). At the end of the cool storage, fruits were maintained for a week at 25 °C (S1 + 7d and S2 + 7d).

The flesh firmness was reduced at the end of cool storage and the soluble solids content significantly increased, for exception of fruits harvested at T2 and stored for 6 months at 0 °C and a week at ambient temperature (S2 + 7d). Some nutritional characteristics such as vitamin C and carotenoids were higher in fruits gathered at T1 but these parameters were strongly influenced by storage, with a general decrease at the end of the long cool storage (6 months). Differently, no influence of long storage was observed in the fruit collected at T2 time. The maintenance for a week at room temperature, after long cool storage, determined an improvement of nutritional characteristics of kiwifruits. In conclusion, fruits harvested at T2 seem to improve their quality after a long storage (6 months) because they reach nutritional values similar or higher than those recorded in fruits at the harvest time. In spite of these positive results, these fruits showed a reduction in organoleptic characteristics which could negatively influence the fruit marketing. The obtained results underline the important role of the pre- and post-harvest factors on the qualitative and nutritional characteristics of kiwifruits.

Keywords: Antioxidant capacity; Ascorbic acid; Harvest time; Kiwifruit; Phenols; Storage

1. Introduction

Fruit and vegetables contain significant levels of biologically active components with physiological and biochemical functions which benefit human health. In the last few years, food has assumed the status of "functional food"; in fact, it must satisfy the nutritional requirements and, at the same time, it can bring several physiological benefits, such as the prevention of important pathologies. More than any other type of food, fruit benefits from having a healthy halo, a halo that's being constantly burnished by a steady stream of news about fruit's intrinsic health benefits. In fact, fruit is an excellent food characterised by a low content of calories and a high amount of antioxidant substances which are able to prevent a wide range of pathologies, such as cancer, cardio-vascular diseases, and degenerative illnesses connected to the aging processes. For this reason, fruit and vegetables represent a major source of dietary antioxidants. Kiwifruit is small caloric and has high amounts of vitamin C (Wills & Greenfield, 1981); it also contains significant amounts of pigments, including chlorophylls and carotenoids. In kiwifruits, the vitamin C content is higher than that determined in orange, strawberry, lemon and grape-fruits and Beever and Hopk-

* Corresponding author. Tel.: +39 50 2216613; fax: +39 50 2216630.
E-mail address: guidilu@agr.unipi.it (L. Guidi).

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irik (1990) showed that vitamin C content in kiwifruit was ten fold higher than the same content found in apple and peach. In particular, some authors (Lintas, Adorisio, Cappelloni, & Monastra, 1991; Selman, 1983) recorded that vitamin C concentration in fruits of cv. 'Hayward' changed from 37 to 200 mg/100 g of fresh weight. In addition, kiwifruit is a nutritious fruit distinguishable from other fruits by the attractive green colour of their flesh; this colour is mainly due to chlorophylls *a* and *b* (Fuke, Sasago, & Matsuoka, 1985; Possingham, Coote, & Hawker, 1980).

The content of phytochemical substances is influenced by numerous factors such as ripening time, genotype, cultivation techniques, climatic conditions that occur during the pre-harvest period but also the operations carried out during the post-harvest storage are very important (Lee & Kader, 2000). Esti et al. (1998) have observed that the vitamin C content of kiwifruit depends on genotype, ripening degree, storage and the analysis method utilised. Indeed, these authors showed that the ascorbic acid (AA) content in kiwifruit samples from genotypes of *Actinidia chinensis* (Planch) var. chinensis was higher than the typical mean content in *Actinidia deliciosa* (A. Chev) cv. 'Hayward'. Generally, Imeh and Khokhar (2002) underlined various factors (agronomic, genomic, pre- and post-harvest conditions and processing) which may affect the chemical composition of plant foods and they may have a significant role in determining the phenolic composition and the bioactivity of these compounds.

Maturity stage is another important factor that influences the compositional quality of fruit and vegetables. In fact, during fruit ripening, several biochemical, physiological and structural modifications happen and these changes determine the fruit quality attributes. Harvesting at the proper maturity stage is essential for optimum quality and often for the maintenance of this quality after harvest and storage. In fact, storage can influence the quality indices and nutritional content of fresh fruit. During post-harvest storage of horticultural crops, important changes in antioxidant status can occur (Ayala-Zavala, Wang, Wang, & Gonzales-Aguilar, 2004). Temperature management is the most important tool to extend shelf-life and maintain quality of fresh fruit and vegetables (Lee & Kader, 2000).

In relation to the importance of phytochemicals and antioxidant power for functional aspect of kiwifruits, the aim of this work was to evaluate the influence of harvest time and storage on several kiwifruit quality attributes, such as vitamin C content, carotenoid content, antioxidant capacity and total phenols. Moreover, the flesh firmness (FF) and the soluble solids content (SSC) of fruits have been estimated.

2. Materials and methods

2.1. Materials

Trials were conducted in the 2005 growing season on fruits from a commercial kiwifruit orchard (*Actinidia deliciosa*, cv. "Hayward") located at Rigoli (Pisa, Italy), where vines were trained as a free palmette.

The quality indices were evaluated at two different time of harvest [November 17 (T1) and 24 (T2) 2005] when fruits have reached 8° and 10° Brix, respectively, measured directly in the field on 20 fruit samples randomly collected on the whole canopy of more plants in the orchard. Two other groups, composed by 40 fruits each, were stored at 0 °C, for 2 or 6 months (S1 and S2, respectively). At the end of cold storage, indices were measured in 20 fruits whereas the last 20 fruits were maintained for another week at room temperature (S1 + 7d and S2 + 7d, respectively for the two storage lengthiness). After this period fruits were analysed for their quality indices.

The quality indices measured were soluble solids content, flesh firmness but ascorbic acid, carotenoids and phenolic content as well as total antioxidant capacity were also determinded.

2.2. Antioxidant capacity

The method used to test the antioxidant capacity measured the iron-reducing capacity of a pool of antioxidant substances of the extracts of the kiwifruit. The FRAP method is developed to measure the ferric reducing ability of a fruit extract at low pH. The FRAP assay treats the antioxidant as reductants in a redox-linked colorimetric reaction. An intense blue colour is formed when the ferric-tripyridyltriazine (Fe^{3+}-TPZ) complex is reduced to the ferrous form at 593 nm. The Fe^{2+} makes a complex with 2,2′-dypirydil. The procedures used were reported in Tavarini, Degl'Innocenti, Pardossi, and Guidi (2007). The final value of antioxidant capacity was expressed as mmol Fe^{2+}/100 g FW.

2.3. Extraction and analysis of carotenoids

The absorbance at $\lambda = 470$ nm of extracts in acetone (80%) was determined (Porra, Thompson, & Kriedman, 1989). The pigment concentrations were expressed in μg/100 g FW.

2.4. Extraction and analysis of vitamin C

Procedures used were as described by Degl'Innocenti, Guidi, Pardossi, and Tognoni (2005) based on the method of Kampfenkel, Van Montagu, and Inze (1995) for the spectrophotometric determination of ascorbic acid (vitamin C). The Vitamin C content was expressed as mg/100 g FW.

2.5. Extraction and analysis of phenols

Phenols were analysed by using the method reported by Dewanto, Adom, and Liu (2002) based on the method of Folin-Ciocalteau, that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex. The values were expressed as mg gallic acid/100 g FW.

2.6. Flesh firmness

The penetration test regards the measure of the necessary strength to imprint to the flesh-fruit a metal point of a dynamometer, to an established distance. The measure was performed on two opposite faces of the equatorial zone by using a digital penetrometer installed on a driving column equipped with an 8 mm probe (Model 53205, TR, Forlì, Italy). Measurements were carried out on a flat surface by removing the skin from two side of the fruits.

2.7. Soluble solids concentration

Soluble solids concentration (SSC) was estimated by the mean of two refractometer readings taken for the juice. A digital refractometer (Model 53011, TR, Forlì, Italy) was used. Measurements were carried out at the same sites as FF and was expressed as °Brix.

2.8. Statistical analysis

Data were subjected to a repeated measures two ways analysis of variance (ANOVA) to determine the significance of differences between treatments which consisted in harvest time and storage. Least significant difference at 5% level (LSD) was calculated to compare differences between means following a significant ANOVA effect. The comparison was carried out to evidence differences among different storage modality. For analysis of correlation between antioxidant capacity and antioxidant substances, the regression analysis was carried out.

3. Results and discussion

3.1. Quality indices

Soluble solids content and firmness of kiwifruit at the two harvest times and at the end of storage have been reported in Table 1. The flesh firmness significantly decreased during storage independently to the harvest time; the lowest values were registered in fruits maintained at ambient temperature for a week after 6 months of cool storage (Table 1). Crisosto and Kader (1999) reported that late harvest kiwifruit retain their flesh firmness during storage better than early harvested fruits. Also in our case, fruits harvested at T2 maintained a better flesh firmness in comparison to T1 fruit ($P < 0.05$). In comparison with data obtained from Crisosto and Kader (1999), it is important to underline that in our case the first harvest T1 was carried out when fruit was near the physiological maturity stage (8 °Brix). In addition to, in S1 and S2, fruit samples clearly showed symptoms of softening; these symptoms enhanced after a week at ambient temperature. This confirms the influence of temperature on the quality indices of fruit as reported also by other authors. In fact, Marsh et al. (2004) showed that kiwifruit stored at different temperatures softened; in particular, these authors found that fruits held at 10° and 4° were characterised by a softening faster than fruits held at 0 °C.

Soluble solids content of kiwifruit at harvest is considered an index of fruit maturity and an increase in SSC corresponds to a conversion of starch to soluble sugars (MacRae, Bowen, & Stec, 1989). In fruits collected at T1 and stored at 0 °C for 2 months, the SSC significantly increased compared with SSC values determined at harvest time (Table 1). The further conservation (S1 + 7d, S2 and S2 + 7d) of fruits did not influence the SSC which remained significantly similar to the values reached at the end of the cool storage period for 2 months (Table 1). A similar behaviour was observed in fruits collected at T2. Also the cool storage for 6 months did not influence the SSC and, in this case, no variations were registered in the following week at ambient temperature. The SSC of kiwifruit is often believed to be linked to consumer test preference, although a close linkage has not been unequivocally demonstrated or registered (McGlone & Kawano, 1998). Fruits above 12 °Brix are generally considered more acceptable to consumers (Stec, Hodgson, MacRae, & Triggs, 1989). Additionally, fruit with a high SSC at harvest, store well and have a satisfactory flavour when eating-ripe (Beever & Hopkirik, 1990).

3.2. Antioxidant capacity

The antioxidant capacity was not influenced by the harvest time (Fig. 1). In fruits collected at T1 the antioxidant

Table 1
Firmness (kg) and solid soluble content (SSC) in kiwifruits harvested in two different times (T1: 17-11-2005, and T2: 24-11-2005) and stored for 2 (S1) and 6 months (S2) at 0 °C

	Firmness (kg)		SSC (°Brix)	
	T1	T2	T1	T2
Harvest	5.89 ± 0.80 a	4.68 ± 0.19 a	8.3 ± 0.89 b	10.4 ± 0.13 b
S1	1.30 ± 0.09 b	1.44 ± 0.10 b	14.3 ± 0.40 a	13.2 ± 0.26 b
S1 + 7 d	1.26 ± 0.56 b	1.36 ± 0.71 b	14.0 ± 0.30 a	14.0 ± 0.67 a
S2	0.92 ± 0.04 bc	1.04 ± 0.11 bc	14.3 ± 0.43 a	13.6 ± 0.23 b
S2 + 7 d	0.32 ± 0.01 c	0.41 ± 0.04 c	14.6 ± 0.98 a	13.8 ± 0.34 b

At the end of the two period of cold storage, fruit were maintained for 7 days at 25 °C (S1 + 7d and S2 + 7d). Each value represents the mean of 5 replicates ± standard deviation. For the two harvest time T1 and T2, means followed by the same letters are not significantly different for $P = 0.05$.

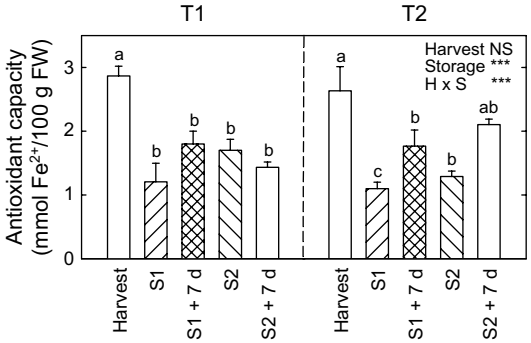

Fig. 1. Antioxidant capacity determined by FRAP assay in kiwifruits harvested in two different times (T1: 17-11-2005, and T2: 24-11-2005) and stored for 2 (S1) and 6 months (S2) at 0 °C. At the end of the two period of cold storage, fruits were maintained for 7 days at 25 °C (S1 + 7d and S2 + 7d, respectively). The bars represent the mean of 3 replicates with standard deviation. For the two harvest time T1 and T2, means followed by the same letters are not significantly different for $P = 0.05$. In the graph results obtained by the two way ANOVA test with harvest time and storage as variability factors are reported.

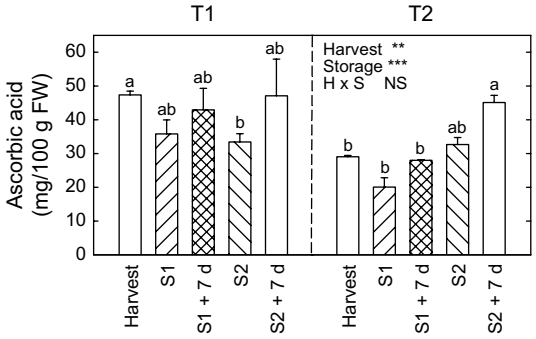

Fig. 2. Ascorbic acid content in kiwifruits harvested in two different times (T1: 17-11-2005, and T2: 24-11-2005) and stored for 2 (S1) and 6 months (S2) at 0 °C. At the end of the two period of cold storage, fruits were maintained for 7 days at 25 °C (S1 + 7d and S2 + 7d). The bars represent the mean of 3 replicates with standard deviation. For the two harvest time T1 and T2, means followed by the same letters are not significantly different for $P = 0.05$. In the graph results obtained by the two way ANOVA test with harvest time and storage as variability factors are reported.

capacity decreased significantly after 2 months of cool storage (S1); then values of FRAP did not change furthermore. The FRAP values of fruits harvested at T2 significantly decreased after 2 months of cool storage too, but the antioxidant capacity significantly increased when these fruits were maintained for a week at ambient temperature (Fig. 1). The negative effect of cool storage in antioxidant capacity of kiwifruits was detected, also after 6 months of storage at 0 °C. Also in these fruits stored for a long period (6 months) an increase in FRAP values was observed at the end of their maintenance at 25 °C (S2 + 7d). To note, however, that the highest values of antioxidant capacity in kiwifruits had been recorded at the harvest, independently from the time of harvest. These results suggested that the cool storage negatively influenced the total antioxidant capacity of kiwifruits. Shivashankara, Isobe, Al-Haq, Takenaka, and Shina (2004) determined the antioxidant capacity in Irwin mango fruit before and after the storage period and they found that the antioxidant capacity remained unchanged up to 20 days of the storage period and decreased thereafter. Also Connor, Luby, Hancock, Berkheimer, and Hanson (2002) determined a reduction of the antioxidant capacity in 9 cultivars of blueberry fruits during cold-temperature storage (3–5 weeks). These authors found an increase in the antioxidant capacity only in the first post-harvest interval (up to 3 weeks) for 3 cultivars. The fruit antioxidant capacity is attributable certainly to the phytochemical content and, in fact, Connor et al. (2002) linked the increase in antioxidant capacity to the phenol content recorded in the first phase of storage. On the other hand, also Shivashankara et al. (2004) linked the reduction after 20 days of storage mainly to the strong relationship with ascorbic acid and they suggested that an increase in antioxidant capacity during low-temperature storage may be possible only in fruit in which the contribution of total phenolics is greater than that of the ascorbic acid.

3.3. Vitamin C

In fruit collected at both harvest time (T1 and T2), the ascorbic acid content (AA) did not show differences in the first post-harvest interval (S1 and S1 + 7d) in comparison with the values at harvest (Fig. 2). In kiwifruit collected at T1, the AA significantly decreased at the end of the long storage (S2) and slightly increased again after a week to ambient temperature (S2 + 7d) (Fig. 2). The AA of kiwifruit harvested at T2 did not change at the end of the long storage (S2) and increased during the following week at ambient temperature (S2 + 7d). Generally, freshly fruits contain more vitamin C than those cool-stored (Lee & Kader, 2000) and, moreover, the loss of vitamin C is extremely variable among different fruits and vegetables. The harvest time significantly influenced the ascorbic acid concentration ($P < 0.01$; Fig. 2) and in fact the value registered at T1 was significantly higher than that at T2. The accumulation of AA during ripening depends on type of fruit; Lee and Kader (2000) reported that AA content increased with ripening in apricot, peach and papaya, but decreased in apple and mango. Generally, when fruits become overripe, vitamin C content declines, concurrently with the degradation of fruit tissues (Kalt, 2005).

It is well known that kiwifruit, as well as *Citrus* fruits, are excellent sources of vitamin C (Nishiyama et al., 2004). However, the antioxidant capacity of kiwifruit is not so high as compared with other fruits. Several authors reported, for example, that strawberries have greater antioxidant capacity (two to eleven fold) than apple, peach, pear, grape, tomato, orange or kiwifruit (Halvorsen et al., 2002; Wang, Cao, & Prior, 1996).There is a controversial debate in the literature about the influence of vitamin C on the antioxidant capacity of fruits or vegetables (Guo et al., 2003). On the other hand, it is also known that fruits with high antioxidant capacity generally contain

more antioxidants and most of these antioxidants has been showed to be phenolic compounds and in particular flavonoids (Connor et al., 2002; Guo et al., 2003; Wang et al., 1996). Even Proteggente et al. (2002) reported that the highest antioxidant capacity found in fruits such as strawberry, raspberry and red plum is attributable essentially to the higher content in anthocyanins.

3.4. Phenol content

The interaction between harvest time and storage had no significant influence on the phenol content of kiwifruit, but the influence of the two variability factors separately was significant (Fig. 3). Phenols did not change in fruit collected at T1 and stored for 2 months at 0 °C (S1) and during the following week at 25 °C (S1 + 7d). A significant rise was observed in these fruits after the long storage (S2) which further increased after a week at ambient temperature (S2 + 7d). The same pattern was observed at the end of storage in fruits collected at T2 too (Fig. 3). In a study about fresh-cut fruits versus whole fruits during storage, Gil, Aguayo, and Kader (2006) found that during 9 days of storage no significant changes occurred in phenol content in kiwifruits and no difference was determined between slices and whole fruits. Generally, phenol content may either increase or decrease in fruits and vegetables depending on the storage conditions (Kalt, 2005).

3.5. Carotenoid content

In Fig. 4 the carotenoid content at harvest and during storage conditions are shown. The harvest time significantly influenced the carotenoid content: higher values of carotenoids had been recorded in fruits at T1 (Fig. 4). The carotenoid content in T1 and T2 harvested kiwifruit increased after 2 months of cool storage and after a week to ambient temperature (Fig. 4), whereas after 6 months of cool storage, fruits harvested at T1 and T2 showed a significant decrease in carotenoid content. A little increase in carotenoids was observed in fruit collected at T1 and maintained for a week to ambient temperature (S2 + 7d). The obtained results showed that a long storage at 0 °C determined a significant loss of carotenoid content of kiwifruit collected both T1 and T2; then this indicates that harvest time is an important factor able to influence the maintenance of total carotenoids during storage. In fact, kiwifruit harvested at T2 showed lower values at harvest than during storage.

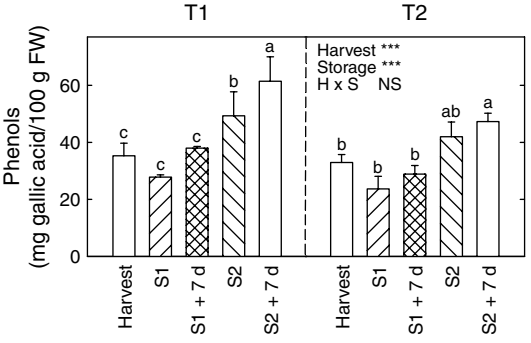

Fig. 3. Total phenol content in kiwifruits harvested in two different times (T1: 17-11-2005, and T2: 24-11-2005) and stored for 2 (S1) and 6 months (S2) at 0 °C. At the end of the two period of cold storage, fruits were maintained for 7 days at 25 °C (S1 + 7d and S2 + 7d). The bars represent the mean of 3 replicates with standard deviation. For the two harvest time T1 and T2, means followed by the same letters are not significantly different for $P = 0.05$. In the graph results obtained by the two way ANOVA test with harvest time and storage as variability factors are reported.

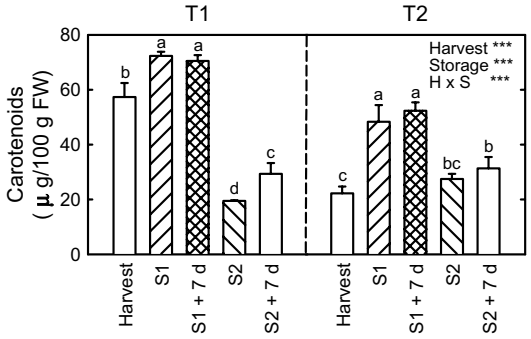

Fig. 4. Total carotenoids in kiwifruits harvested in two different times (T1: 17-11-2005, and T2: 24-11-2005) and stored for 2 (S1) and 6 months (S2) at 0 °C. At the end of the two period of cold storage, fruits were maintained for 7 days at 25 °C (S1 + 7d and S2 + 7d). The bars represent the mean of 3 replicates with standard deviation. For the two harvest time T1 and T2, means followed by the same letters are not significantly different for $P = 0.05$. In the graph results obtained by the two way ANOVA test with harvest time and storage as variability factors are reported.

3.6. Correlation between fruit constituents and total antioxidant capacity

To highlight the influence of the phytochemical constituents on antioxidant capacity in kiwifruit, we determined the correlation between the FRAP values and different antioxidant substances (vitamin C, carotenoids and phenols). Also for correlation analysis we considered the two harvest time separately. From the statistical analysis, only one correlation resulted significant and positively correlated (Fig. 5) AA content was positively correlated with the total antioxidant capacity only in fruit collected at T1. This result also agreed with the contribution of AA to total antioxidant capacity, determined in these fruits which corresponded to a value of about 40%. These results suggest that, in kiwifruits, vitamin C contributed to antioxidant capacity much more than others antioxidant constituents, such as phenols or carotenoids. On the other hand, kiwifruit is characterised by a high content of vitamin C and a small amounts of phenolics (Gil et al., 2006).

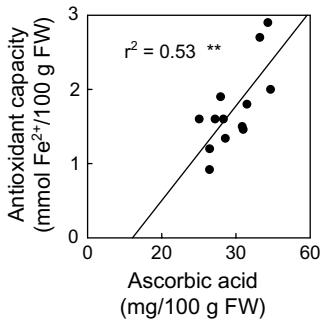

Fig. 5. Linear regression between values of total antioxidant capacity and ascorbic acid content in kiwifruits collected at the time T1 and stored for 2 or 6 months at 0 °C (S1 and S2) and for a week at 25 °C (S1 + 7d and S2 + 7d, respectively). $^{**}P < 0.01$.

4. Conclusion

The obtained results underline the important role of the pre- and post-harvest factors on the qualitative and nutritional characteristics of kiwifruit. Among the pre-harvest factor, certainly the harvest time influenced organoleptic and nutritional characteristics of kiwifruit. SSC increased in fruit collected at T2 but some nutritional characteristics such as vitamin C and carotenoids were higher in fruits gathered at T1, while harvest time did not induce any change in antioxidant capacity and phenol content.

The cool storage had a different effect in relation to the organic compound considered. In fact, cool storage, and in particular its duration, influenced nutritional characteristics in kiwifruits even if is not possible to identify a unique behaviour for the organic compounds determined here.

Cold storage conditions significantly increased total phenolics and this can be attributed to changes occurring in phenol metabolism during storage, as well as the increase of phenylalanine ammonia lyase (PAL). It is known as PAL has been found to be associated with post-harvest disorders induced after prolonged storage at low-temperature (Martinez-Tellz & Lafuente, 1997).

The most interesting result was the decrease in AA observed in T1 harvested fruits after 6 months of cool storage and this result agrees with the correlation found between the content of this molecule and antioxidant capacity. The temperature upon storage is the most important factor in the post-harvest life of fresh produce because of its dramatic effects on rates of biological reactions (Li & Kader, 1989). Water loss during storage is another important cause of fruit deterioration. In particular, loss of fresh weight (water loss) can also speed up ascorbic acid degradation.

Interestingly, it appears the effect determined by the maintenance of fruits at room temperature after cool storage, which sometimes improved the nutritional characteristics of fruit as well as the phenol and carotenoid content in T1 and T2 harvested fruits and AA only in fruit collected at T2 time. In conclusions, fruits harvested at T2 seem to be more suitable for a long storage (6 months) because reach nutritional values also higher than those recorded in fruits at the moment of harvest. However, these fruits were also characterised by a reduction in organoleptic characteristics such as flesh firmness, a parameter that can compromise the fruit marketing.

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