Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 91 (2005) 113–121
Food Chemistry
www.elsevier.com/locate/foodchem

Effects of temperature on the chemical composition and antioxidant activity of three strawberry cultivars

Beatriz Rosana Cordenunsi, Maria Inés Genovese, João Roberto Oliveira do Nascimento, Neuza Mariko Aymoto Hassimotto, Ricardo José dos Santos, Franco Maria Lajolo *

Laboratório de Química, Bioquímica e Biologia Molecular de Alimentos, Departamento de Alimentos e Nutrição Experimental, FCF, Universidade de São Paulo, Av. Prof. Lineu Prestes 580, Bloco 14, 05508-900 São Paulo, SP, Brazil

Received 8 March 2004; received in revised form 27 May 2004; accepted 27 May 2004

Abstract

Three strawberry cultivars (Dover, Campineiro, and Oso Grande), grown in the same commercial plantation, were harvested at the ripe stage and stored at 6, 16 and 25 °C, for 6 days. During this period, chemical composition and antioxidant activity were evaluated. Results showed an increase in total soluble sugars, anthocyanin and vitamin C contents, indicating that a new biosynthesis had taken place during storage. Low temperature negatively affected anthocyanin and vitamin C accumulation, and positively affected soluble sugars, while flavonols, ellagic acid and total phenolic contents remained almost the same or even decreased at all temperatures. Despite differences in anthocyanin content between varieties and its increase during storage (higher with increasing temperature), there was no difference in the antioxidant activity between cultivars, which decreased after harvesting, independently of the temperature of storage. Variations in the proportion dehydroascorbic acid/ascorbic acid (DHA/AA) showed that there were differences between cultivars concerning adaptation of the fruit to low temperatures. The data obtained here indicate that cold storage is an effective way to maintain strawberry quality, but a compromise between sensorial and nutritional values can be achieved at 16 °C, for all the cultivars.

Keywords: Strawberries; Storage; Anthocyanins; Soluble sugars; Antioxidant activity; Vitamin C; Flavonols

1. Introduction

Strawberries are a good source of ascorbic acid (AA), anthocyanins and flavonols and, among the fruits, they have one of the highest antioxidant activities evaluated by oxygen radical absorbance capacity (Cordenunsi, Nascimento, Genovese, & Lajolo, 2002; Wang, Cao, & Prior, 1996). Like other fruits, strawberries can be consumed "in natura", which turns out to be advantageous to consumers since there are no nutritional losses due to processing. On the other side, the preference for fresh fruits is challenging because they have a very short shelf-life, due to their sensitivity to fungal attack and excessive texture softening caused by the natural ripening process.

To circumvent the losses associated with handling and storage of strawberry, and other small fruits, some postharvest conditions, such as low temperature or high CO_2 concentration, as well as controlled atmosphere or a combination of both processes, are widely used to extend the shelf-life (Gil, Holcroft, & Kader, 1997; Pelayo, Ebeler, & Kader, 2003). Since there are some injuries associated with postharvest handling (Manning, 1996), fruit quality is evaluated in terms of its main sensorial attributes, in order to maintain consumer acceptance.

* Corresponding author. Tel.: +55-11-3818-3647; fax: + 55-11-3815-4410.
E-mail address: fmlajolo@usp.br (F.M. Lajolo).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.05.054

In this way, organoleptic parameters, such as texture, freshness and colour are considered important, but the nutritional or functional issues are not considered. As a result, there are few studies focussing on the effects of strawberry storage conditions on nutritional parameters.

Controlled atmosphere has the undeniable benefit of controlling postharvest decay of fruits, but a CO_2-enriched atmosphere with low O_2 concentration can affect total ascorbic acid and anthocyanin contents adversely, with a negative consequence in fruit colour and nutritional value (Holcroft & Kader, 1999). It is already known that anthocyanin synthesis continues after harvest and also low temperature storage (Kalt, Forney, Martin, & Prior, 1999), but it is inhibited in fruits stored in high CO_2 concentrations. Holcroft and Kader (1999) found a negative effect of the atmosphere on the anthocyanin concentration and on the activities of the key enzymes of the anthocyanin synthesis pathway: phenylalanine ammonia lyase and UDP-glucose: flavonoid glucosyltransferase. Also Kalt et al. (1999) concluded that low temperatures could affect anthocyanin synthesis during storage of small fruits, inclusive of strawberries.

Like anthocyanins, the amount of AA is also dependent on the strawberry cultivar and ripening degree, although the average content (60 mg/100 g) is high enough to consider strawberry as one of the richest sources of AA among fruits (Cordenunsi et al., 2002). To retain the initial AA content during storage, temperature management is the most important factor to be taken into account. In this respect the speed of lowering the temperature after harvest and the temperature of storage are crucial (Lee & Kader, 2000). Ascorbic acid is the predominant form of vitamin C present in fruits, and the primary oxidation product. L-Dehydroascorbic acid (DHA) is also important because it also has biological activity. The average content of DHA in fruits is less than 10% of total vitamin C content and, to our knowledge, only one report indicated an increase of the DHA/AA ratio during storage (Lee & Kader, 2000). Since the oxidized form is more prone to decomposition, leading to the loss of biological activity, the changes in AA forms are important in both, technological and nutritional terms.

There is a controversy in the literature about the influence of vitamin C content on the antioxidant capacity of vegetables. On the other hand, there is a consensus that the antioxidant capacity is directly correlated with phenolic compounds, especially flavonoids (Connor, Luby, Hancock, Berkheimer, & Hanson, 2002; Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999; Wang et al., 1996). Although the antioxidant compounds have an important role in human health, only a few reports have focussed on their changes in fresh fruit stored at low temperature.

Temperatures around 0 °C are considered the best for strawberry storage because they cause few changes in quality. However, the commercialization and post-market storage usually occur at higher temperatures. These higher temperatures can affect, not only the strawberry shelf-life, but also its nutritional value, in terms of soluble sugars, vitamin C and antioxidant compounds. This study presents the temperature responses of three strawberry cultivars in relation to nutritional parameters and antioxidant capacity.

2. Material and methods

2.1. Material

Strawberry fruits (*Fragaria ananassa* Duch.), of the cultivars Dover, Campineiro (Brazilian cultivar) and Oso Grande, were harvested in the same commercial plantation located in Atibaia (São Paulo State), at the stage 5 (full size 3/4 red). Fruits of each variety were divided in three groups and stored at 6, 16 °C and room temperature (~25 °C), for 1–6 days. Samples of at least 40 fruits of each period were picked, made into pieces, immediately frozen in liquid nitrogen and stored at −80 °C. At the time of analysis, samples were thoroughly homogenized by powdering in liquid nitrogen. The controls corresponded to fruits frozen at the day of harvesting. Sampling of the stored strawberries was limited by the natural senescence of the fruits.

2.2. Carbohydrate determination

Soluble sugars were extracted three times with 80% ethanol at 80 °C. After centrifugation, the supernatants were combined and the ethanol was evaporated under vacuum. The soluble sugar content was analyzed by high pressure liquid chromatography with pulse amperometric detection (HPLC-PAD – Dionex, Sunnyvale, CA, USA), using a PA_1 column (Dionex, Sunnyvale, CA, USA) in an isocratic run of 18 mM NaOH during 25 min. Total soluble sugars were obtained as the sum of glucose, fructose and sucrose values.

2.3. Ascorbic acid determination

Total AA was extracted with metaphosphoric acid (1% w/v) and analyzed by reversed-phase HPLC in a Hewlett–Packard 1100 system coupled to a diode array detector. The column used was a μ-Bondapak (300 mm × 3.9 mm i.d., Waters, Milford, USA), and elution (flow rate of 1.5 ml/min) was performed, under isocratic conditions, with 0.2 M sodium acetate/acetic acid buffer (pH 4.2), monitored at 262 nm. Total AA was estimated

after reduction of DHA with 10 mM 1,4-dithiothreitol (DTT). Dehydroascorbic acid was calculated as the difference between total-AA and reduced form of AA.

2.4. Flavonol, anthocyanin and ellagic acid contents

Extraction of flavonoids was performed as previously reported (Cordenunsi et al., 2002). Frozen samples (~30 g, in duplicate) were extracted three times in cold methanol:water:acetic acid (70:30:5) with a Brinkmann Homogenizer (Polytron® – Kinematica GmbH, Kriens-Luzern, Sweden). The homogenate was filtered under reduced pressure through filter paper (Whatman no 1) and the combined fractions were evaporated under vacuum at 40 °C to ~20 ml in a Rotavapor® RE 120 (Büchi, Flawil, Sweden) and made up to 25 ml with water. An aliquot of 10 ml of the extract was added to a 1 g-polyamide SC6 column (Macherey-Nagel Gmbh & Co. Germany) preconditioned with methanol (20 ml) and water (60 ml). The column was washed with water (20 ml) and further eluted with 40 ml methanol, followed by 40 ml of methanol:ammonia (99.5:0.5). These fractions were evaporated to dryness under reduced pressure at 40 °C, redissolved in methanol (1 ml) and filtered through 0.22 μm PTFE filters (Millipore Ltd., Bedford, USA). Identification and quantification was achieved by using analytical reversed-phase HPLC in a Hewlett–Packard 1100 system with autosampler and quaternary pump coupled to a diode array detector. The column used was a Prodigy 5μ ODS3 reversed phase silica (250 mm × 4.6 mm i.d., Phenomenex Ltd.) and elution solvents were: A, water:tetrahydrofuran: trifluoroacetic acid 98:2:0.1 and B, acetonitrile. Solvent gradient was the same as used by Price, Prosser, Richetin, and Rhodes (1999), except for the separation of acidic flavonols, where the initial % B was 25%, in order to allow separation of ellagic acid from quercetin glucuronide. Samples were injected in duplicate and flavonols were quantified using quercetin and kaempferol (Sigma Chemicals Co., St. Louis, USA) as external standards. For anthocyanins, standard solutions of pelargonidin chloride (Extrasynthèse, Genay, France) and cyanidin-3-rutinoside (Apin Chemicals Ltd., Abingdon, UK) were used. Ellagic acid was from Sigma Chemicals Co. Calibration was performed by injecting the standard three times at five different concentrations. Results were expressed as mg/100 g fresh weight (FW).

2.5. Total phenolics

Total phenols were determined according to the method of Swain and Hillis (1959), using the Folin–Ciocalteau reagent. Results were expressed as mg catechin/100 g FW.

2.6. Antioxidant activity

Powdered frozen samples (~1 g, in triplicate) were extracted twice in cold 70% aqueous methanol containing 5% acetic acid (10 ml portion each time) with a Brinkmann Homogenizer (Polytron® – Kinematica GmbH, Kriens-Luzern, Sweden). The homogenate was centrifuged at 10,000*g* for 10 min at 4 °C and made up to 25 ml with 70% aqueous methanol containing 5% acetic acid. For the antioxidant assay the extracts were diluted 400 times with the extraction solution. The antioxidant activity was determined according to the β-carotene bleaching method, following a modification of the procedure described by Marco (1968). A 20 μl aliquot of a β-carotene (Sigma Chemicals Co., St. Louis, USA) solution (100 mg/50 ml of chloroform) was added to a 200 ml-Erlenmeyer flask containing 1.0 ml of chloroform, 0.4 ml of linoleic acid and 0.4 ml of Tween 40 (Sigma Chemicals Co., St. Louis, USA), and the chloroform was evaporated to dryness under nitrogen. Oxygenated distilled water (100 ml) was added to the dried mixture, mixing thoroughly. Aliquots (100 μl) of the strawberry extracts or 70% MeOH (as the control) were added to 2.9 ml of this β-carotene solution in cuvettes, and mixed well. The samples were then subjected to thermal autoxidation at 50 °C. The absorbance of the solution at 470 nm was immediately measured after mixture and 2 h oxidation with a Hewlett-Packard 8453 spectrophotometer. Antioxidant activity was calculated as the percent inhibition relative to the control.

2.7. Statistical analysis

Statistical analysis was done by using the Statistica software package version 5.0 (StatSoft, Inc., Tulsa, USA). Differences between means were first analyzed by ANOVA test and then LSD (least significant difference) test ($P < 0.05$).

3. Results and discussion

3.1. Total soluble sugars (TSS)

At the first day of storage the amount of total soluble sugars was under 5 g/100 g (FW) for all cultivars (Fig. 1) and, as can be seen, lower temperatures had a positive effect on the TSS contents. Except for the Dover cultivar stored at 6 °C, the Campineiro and Oso Grande had their TSS increased up 30%, when stored in a cold room. In a previous experiment (Cordenunsi, Nascimento, & Lajolo, 2003), Campineiro fruits kept at 6 °C for a week showed no changes in TSS values, while Dover had an increase of 10% and Oso Grande had a decrease of

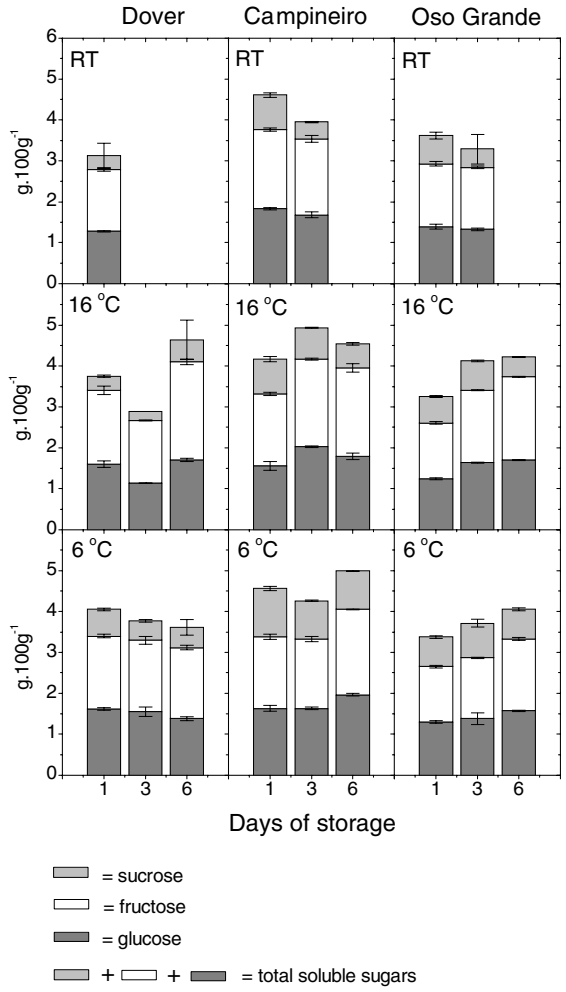

Fig. 1. Sucrose, fructose and glucose contents (g/100 g FW) of cultivars Dover, Oso Grande and Campineiro strawberries during storage at different temperatures (6, 16 and 25 °C). Data presented are means of triplicate assays ± SE.

20% over storage time. Comparable data reported in the literature showed a decrease between of 8% and 16% in TSS of strawberries kept at 5 °C in air (Gil et al., 1997; Pelayo et al., 2003). The apparent discrepancies between the previous and the present data are in agreement with the results reported by Watson (Watson, Wright, McBurney, Taylor, & Linforth, 2002), who found that sugar, citric acid and volatile compounds varied considerably between the fruits and harvests.

The main soluble sugars found in all cultivars were fructose, glucose and sucrose (Fig. 1). At the first day of storage, the fructose to glucose ratio was almost the same (1.1) for all cultivars, the sucrose content being almost half that of fructose. During storage at 16 °C, the fructose/glucose ratio increased for all cultivars, reaching values around 1.4. The higher fructose content at this temperature could be explained by the glucose consumption (by the respiration) which was inhibited at the lower storage temperature (6 °C).

A 20% decrease in the sucrose content was observed for all cultivars, except for 16 °C-Dover, but the amount at the last day of storage was still higher than that described earlier (Cordenunsi et al., 2003). Since sucrose is the primary source of glucose and fructose, the increased amount of these monossacharides could account for the decrease in sucrose level. However, the TSS increase indicates that sucrose synthesis had taken place during cool-storage.

There are three possible carbon sources for soluble sugar synthesis after harvest: starch, organic acids and cell-wall disassembly. Since strawberry fruit has insufficient starch (~0.1%) to support this synthesis, organic acids and cell-wall are the more likely sources. The negative correlation between texture and TSS-increase, observed for Dover and Campineiro during cool-storage, could indicate that cell-wall disassembly plays an important role in sugar accumulation. The increase in xylose content during cold storage of Campineiro and Dover cultivars (Fig. 2) also reinforces this idea. On the other hand, Oso Grande, which is a more stable cultivar in terms of texture (Cordenunsi et al., 2003), showed the highest increase of TSS (up to 30%). Since there were no marked changes in xylose content and the citric acid content decreased (Cordenunsi et al., 2003), this organic

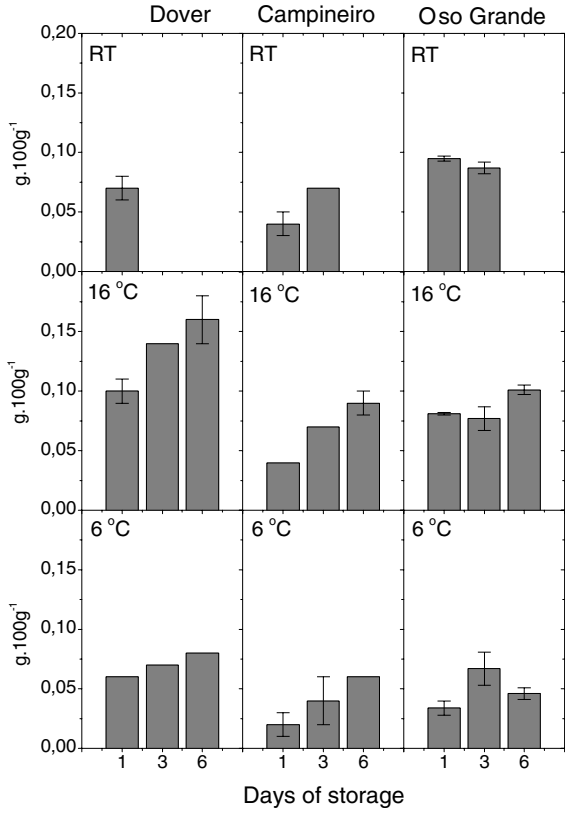

Fig. 2. Xylose content (g/100 g FW) of cultivars Dover, Oso Grande and Campineiro strawberries during storage at different temperatures (6, 16 and 25°C). Data presented are means of triplicate assays ± SE.

acid could be an alternative carbon source for soluble sugar synthesis in cold-stored Oso Grande strawberries.

3.2. Anthocyanins

The amount of anthocyanins is important for the attractiveness and maturity assessment of strawberries. The three cultivars were harvested at full size and when nearly 3/4 of the fruit turned red. As can be seen in Fig. 3, the highest anthocyanin level was detected for Dover under all the storage conditions studied, followed by Oso Grande and Campineiro. The three cultivars accumulated anthocyanin during storage, at rates which increased with increasing temperature. The temperature affected, not only the rate of pigment synthesis, but also the ultimate level of anthocyanins. All the strawberries presented lower amounts of anthocyanin when stored at 6 °C, but Campineiro fruits were the most adversely affected, since the final amount was only 30% of that for fruits stored at room temperature. In this regard, the storage at 16 °C kept the pigment content at levels similar to that of control fruits, in all the cultivars.

Pelargonidin glycosides were the main pigments found (Table 1), corresponding to almost three times the amount of cyanidin glycoside, for Oso Grande, and ten times, for Dover. Only traces of cyanidin were detected in Campineiro fruits of the control group.

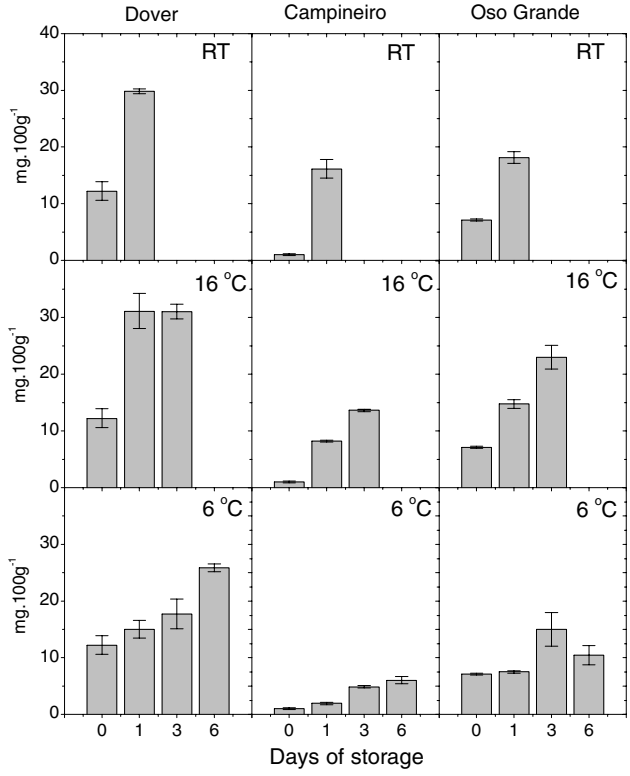

Fig. 3. Anthocyanin content (mg/100 g FW) of cultivars Dover, Oso Grande and Campineiro strawberries during storage at different temperatures (6, 16 and 25 °C). Data presented are means of triplicate assays ± SE.

Table 1
Ratio between pelargonidin and cyanidin glycosides in strawberry cultivars and its change after storage at different temperatures

	Pelargodinin/cyanidin		
	Dover	Campineiro	Oso Grande
Control	10.0	–[a]	2.7
25 °C/2 days	28.4	20.9	5.8
16 °C/3 days	18.1	13.8	5.4
16 °C/6 days	23.6	28.1	5.1
6 °C/1 day	17.3	10.2	3.5
6 °C/3 days	18.4	10.7	5.4
6 °C/6 days	25.8	13.1	4.7

[a] Only traces of cyanidin were detected.

During storage, at all temperatures, there was a significant increase in the ratio between pelargonidin and cyanidin for the three varieties, showing a preferential synthesis of pelargonidin over cyanidin. However, the lower temperature had a negative effect on Campineiro, since the pelargonidin/cyanidin ratio was half that observed at room temperature.

3.3. Flavonols

The total flavonol content (Table 2) was quite similar for the three cultivars and similar to the values previously reported (Cordenunsi et al., 2002), with an initial content ranging from 5.2 (Oso Grande) to 8.2 (Campineiro) mg/100 g FW. Only quercetin and kaempferol derivatives were present and, in general, the amount of quercetin derivatives was more than double that of kaempferol derivatives. Glucuronides (acidic flavonols) were the main derivatives present, corresponding to more than six times the amount of glucosides (neutral flavonols). Contrarily to what was observed for anthocyanins, in general the amount of flavonols did not increase with storage at the temperatures tested. For Dover and Oso Grande, almost the same contents were found after storage whereas, for Campineiro, a decrease in flavonols was observed after 3 days at 6 and 16 °C. However, this difference was not observed for storage at room temperature, in which a content similar to the initial one was found. As a whole, these results indicated that there was no flavonol synthesis after harvesting. For apple, storage at both refrigerator temperature and under controlled atmosphere conditions did not alter flavonoid concentration (van der Sluis, Dekker, de Jager, & Jongen, 2001). Opposite to this, DuPont et al. (DuPont, Mondin, Williamson, & Price, 2000) reported flavonol losses in lettuce stored at 1 °C for seven days, in the range of 7–46%.

3.4. Ellagic acid content

Berries are one of the main sources of ellagic acid in our diet, a hydroxybenzoic acid normally present as a

Table 2
Flavonol and ellagic acid contents (mg/100 g FW) (means ± SD of duplicate assays) of Dover, Oso Grande and Campineiro strawberries during storage at different temperatures (6, 16 and 25 °C)

	Constituent								
	Neutral flavonols		Acidic flavonols		Q Total	K Total	Total flavonols	Free ellagic acid	Total phenolics
	Q	K	Q	K					
Dover									
Control	0.30	0.34	5.03	1.74	5.33	2.08	7.4 ± 0.2a	2.4 ± 0.1a	312 ± 10a
25 °C/2 days	0.41	0.45	4.73	2.20	5.14	2.65	7.8 ± 0.3a	1.8 ± 0.1c	319 ± 28a
16 °C/3 days	0.60	0.65	7.67	1.23	8.27	1.88	10.2 ± 1.0b	3.1 ± 0.3b	308 ± 22a
6 °C/3 days	0.44	0.44	4.75	2.04	5.19	2.48	7.7 ± 0.7a	2.7 ± 0.3ab	319 ± 30a
Campineiro									
Control	0.21	0.15	6.64	1.22	6.85	1.37	8.2 ± 0.3a	2.5 ± 0.4a	299 ± 13a
25 °C/2 days	0.46	0.57	5.03	1.74	5.49	2.31	7.8 ± 0.2a	1.4 ± 0.1c	312 ± 8a
16 °C/3 days	0.32	0.34	4.14	1.04	4.46	1.38	5.8 ± 0.2c	1.8 ± 0.1b	300 ± 24a
6 °C/3 days	0.29	0.28	3.05	0.76	3.34	1.04	4.4 ± 0.2b	1.8 ± 0.0b	243 ± 19b
Oso Grande									
Control	0.36	0.36	3.55	0.96	3.91	1.32	5.2 ± 0.3a	2.4 ± 0.2a	324 ± 12a
25 °C/2 days	0.48	0.32	4.20	1.22	4.68	1.54	6.2 ± 0.3b	1.8 ± 0.2b	345 ± 16a
16 °C/3 days	0.64	0.49	3.55	1.19	4.19	1.68	5.9 ± 0.3b	3.0 ± 0.2c	353 ± 30a
6 °C/3 days	0.49	0.34	4.02	1.13	4.51	1.47	6.0 ± 0.7ab	1.9 ± 0.1b	335 ± 9a

Q, quercitin derivative; K, kaempferol derivative.
Means in the same column with common letters are not significantly different ($P < 0.05$).

polymer (ellagitannin) or glycosylated derivative, which has been shown to have anticarcinogenic action (Parr & Bolwell, 2000; Tomás-Barberán & Clifford, 2000). Häkkinen and Törrönen (2000) reported a 40% decrease in ellagic acid content in strawberries during 9 months of storage in a freezer. The amount of free ellagic acid has been reported to increase with processing and storage of raspberry jams, which was associated with release from ellagitannins caused by thermal treatment and storage (Zafrilla, Ferreres, & Tomas-Barberán, 2001). Our results (Table 2) showed that the three strawberry cultivars presented the same initial free ellagic acid content, of 2.4 mg/100 g FW, slightly higher than the values previously reported by us (Cordenunsi et al., 2002). Although a significant decrease was observed for the Campineiro after storage at the three temperatures, no clear tendency was observed for the other two cultivars, which even showed an increase in free ellagic acid upon storage at 16 °C. At room temperature, the three cultivars presented a lower free ellagic acid content after storage (Table 2). As the amount of free ellagic acid in strawberries is very low relative to that of derivatives and polymerized forms (~5%) (Häkkinen & Törrönen, 2000), it can be concluded that storage did not cause any significant change in these compounds under the conditions used here.

3.5. Total phenolics

The three strawberry varieties presented similar total phenolics contents, of around 300 mg/100 g FW, which were not altered by storage. As can be seen in Table 2, total flavonols and free ellagic acid represented only a small percentage of total phenolics, mainly constituted of *p*-coumaric acids, ellagitannins and glycosylated derivatives of ellagic acid, and anthocyanins (Häkkinen, Kärenlampi, Heinonen, Mykkänen, & Törrönen, 1999). The increase in anthocyanins during storage was not correlated with total phenolics content, which can be due to a higher induction or release of phenolic degradative enzymes or to a higher extractability of these enzymes in tissues that have been stored and whose structures start to degrade.

3.6. Total ascorbic acid

The initial content of total AA ranged from 47 to 80 mg/100 of fresh fruit, in Oso Grande and Campineiro cultivars, respectively (Fig. 4). In all cultivars, the content of the oxidized form of ascorbic acid (DHA, dehydroascorbic acid) was about 8%. Among the cultivars studied, Campineiro has consistently shown the highest total AA content (Cordenunsi et al., 2002, 2003), even considering harvests in two different years (one in 1999 and two in 2000). In this way, besides pre-harvest climactic conditions and postharvest handling procedures (Lee & Kader, 2000), cultivar type can be defined as another important factor affecting vitamin C content.

The variation of total ascorbic acid throughout the storage period (Fig. 4(a)) showed that the vitamin content can be affected not only by the cultivar, but also

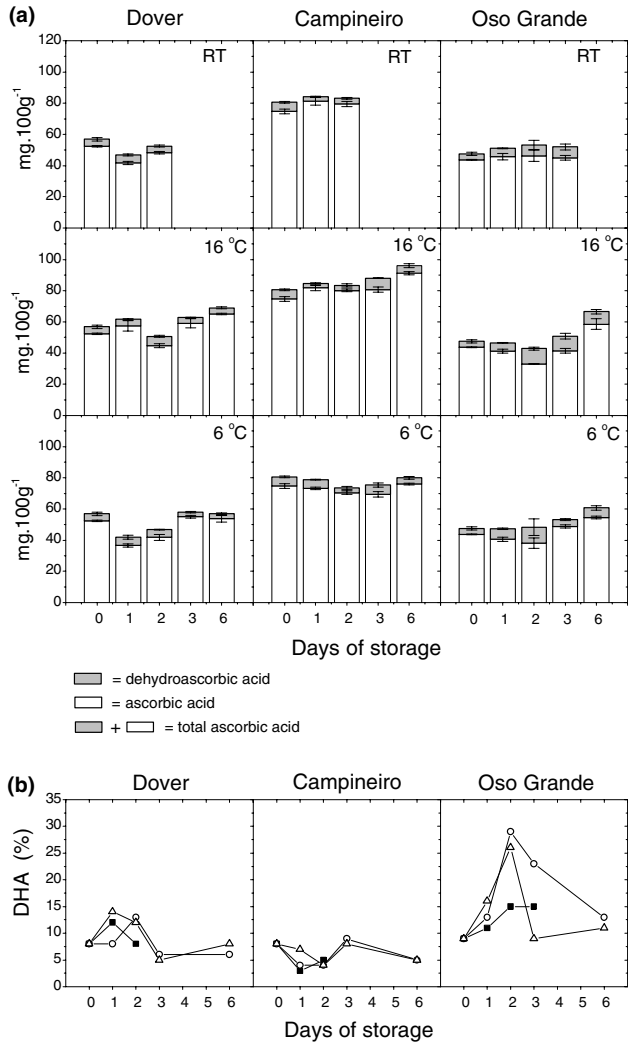

Fig. 4. (a) Total ascorbic acid, dehydroascorbic acid and ascorbic acid contents (mg/100 g FW) of cultivars Dover, Oso Grande and Campineiro strawberries during storage at different temperatures (6, 16 and 25 °C). (b) Profiles of variation of dehydroascorbic acid (%) during storage. Data presented are means of triplicate assays ± SE.

by the temperature. Room temperature-stored Campineiro and Oso Grande fruits showed no significant changes in total AA content during storage, while Dover showed a discrete decrease. When the fruits of the three strawberry cultivars were stored at 16 °C, an increase of at least 10% was detected at the end of storage. However, the same positive effect on AA level can not be achieved by lowering the temperature further.

Storage at 6 °C was an effective way to maintain the initial levels of total AA for additional days, but it did not increase the vitamin content significantly. In this respect, Oso Grande cultivar seemed to be less sensitive to this temperature change, while Dover fruits showed a discrete diminution in AA before recovering to the initial levels. The profiles of DHA (Fig. 4(b)) suggest that there are some differences in the adaptation to the lower temperature, since the DHA content (on a total-AA basis) increased to up 30% for Oso Grande at day 2 at cold temperature, while Dover fruits had a more constant DHA/AA proportion. Althought the results can be taken as an evidence of an operative redox system (AA/DHA) acting during cold storage, there seems to be no fruit-specific pattern of total AA or DHA accumulation, but a cutlivar-specific one.

The fluctuations in the profiles of total-AA and AA contents (Fig. 4) showed that ascorbic acid synthesis took place during the storage period and synthesis was affected by lowering the temperature. There are reports (Lee & Kader, 2000; Wills, Wimalasiri, & Greenfield, 1984) of the positive influence of low temperature in the maintenance of vitamin C content during storage of fruits and vegetables, but to our knowledge, there is no mention of AA synthesis during cold-storage. In a previous work (Cordenunsi et al., 2003), storage at 6 °C caused a 50% decrease of the total AA content in all strawberry cultivars after 6 days. As we found for soluble sugars, vitamin C content at harvest time and its changes during storage seem to be dependent on cultivar, besides cultural practices, light intensity, and climatic conditions.

3.7. Antioxidant activity

Besides anthocyanins, other flavonoids, phenolic acids and vitamins can contribute to the protective effect against oxidative damage to cells. Since the antioxidant activity of individual dietary compounds cannot always be evaluated, the determination of the total antioxidant activity allows a more realistic evaluation of the potential protective effect of a food. The three cultivars of strawberries presented similar antioxidant activities, probably reflecting similar total phenolic contents. However, antioxidant activities decreased upon storage, from 9 to 30% (Table 3). Samples of Campineiro strawberries stored for 6 days at 6 or 16 °C presented a further reduction of antioxidant activity compared to those stored for 3 days.

These data showed that the increase in anthocyanin content and total AA did not result in a proportional increase of the antioxidant activity, as expected. For apples stored both at refrigerator temperature and under controlled atmosphere conditions, no changes in antioxidant activity or flavonoid concentration were detected (van der Sluis et al., 2001), but there was a decrease in antioxidant activity of samples of fresh-cut spinach stored at 10 °C (Gil, Ferreres, & Tomás-Barberán, 1999). In this case, the decrease in antioxidant activity was associated with a higher content of dehydroascorbic acid and a decrease in ascorbic acid, since there were no changes in flavonoid amounts.

The results presented here suggest that the adaptation to the cold environment is a challenging condition to the

Table 3
Total antioxidant activity (% inhibition) of strawberry varieties and its change after storage at different temperatures

	Dover	Campineiro	Oso Grande
Control	55.2 ± 1.3a	56.6 ± 2.6a	51.7 ± 3.6a
25 °C/2 days	50.2 ± 1.8c	49.3 2.8b	46.1 ± 0.6b
16 °C/3 days	47.9 ± 3.7c	45.3 ± 5.2bc	47.2 ± 2.0ab
16 °C/6 days	–	40.5 ± 2.3c	–
6 °C/3 days	39.9 ± 3.8b	45.6 ± 2.3b	43.9 ± 3.6b
6 °C/6 days	–	38.0 ± 3.2c	–

Results are expressed as means ± SD ($n = 3$).
Means in the same column with common letters are not significantly different ($P < 0.05$).

redox state of the tissue. In this way, the increase in AA content and the maintenance of DHA/AA proportion would be at the expense of reducing compounds, other than anthocyanins.

4. Conclusions

As discussed above, lowering the storage temperature is an effective way to extend the strawberry shelf-life, maintaining the fruits edible for additional days. However, temperature can also affect some ripening-related processes, which in turn can improve or decrease both sensorial and nutritional value.

As noted before, an increase in TSS and fructose-glucose ratio took place during the storage at 16 °C, but cultivar-specific adverse effects can occur at lower temperatures since, for Dover fruits, a decrease in total sugars was observed. The contents of flavonols, ellagic acid and total phenolics were not affected by the temperature lowering, while anthocyanin biosynthesis was delayed. The final anthocyanin content of the more sensitive cultivar kept at 6 °C was only one third of that achieved at room temperature. Ascorbic acid was also affected by temperature. The AA synthesis was favoured at 16 °C but an additional decrease in temperature did not improve the final amount of this vitamin.

Based on these data, it is possible to conclude that cold storage is an effective way to maintain strawberry qualities, but the best compromise between sensorial and nutritional value can be achieved at 16 °C, regardless of the cultivar. This finding is not only of sensorial or health relevance, but also economical, because of the additional costs involved in the maintenance of lower temperatures.

Acknowledgements

We are grateful to FAPESP for financial support. We wish to acknowledge the technical assistance of Marcia Morais, Alberto Bernal and Lucia Justino da Silva.

References

Connor, A. M., Luby, J. J., Hancock, J. F., Berkheimer, S., & Hanson, E. J. (2002). Changes in fruit antioxidant activity among blueberry cultivars during cold-temperature storage. *Journal of Agricultural and Food Chemistry, 50*, 893–898.

Cordenunsi, B. R., Nascimento, J. R. O., Genovese, M. I., & Lajolo, F. M. (2002). Influence of cultivar on quality parameters and chemical composition of strawberry fruits grown in Brazil. *Journal of Agricultural and Food Chemistry, 50*, 2581–2586.

Cordenunsi, B. R., Nascimento, J. R. O., & Lajolo, F. M. (2003). Physico-chemical changes related to quality of five strawberry fruit cultivars during cool-storage. *Food Chemistry, 83*, 167–173.

DuPont, M. S., Mondin, Z., Williamson, G., & Price, K. R. (2000). Effect of variety, processing, and storage on the flavonoid glycoside content and composition of lettuce and endive. *Journal of Agricultural and Food Chemistry, 48*, 3957–3964.

Gil, M. I., Holcroft, D. M., & Kader, A. A. (1997). Changes is strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. *Journal of Agricultural and Food Chemistry, 45*, 1662–1667.

Gil, M. I., Ferreres, F., & Tomás-Barberán, F. A. (1999). Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. *Journal of Agricultural and Food Chemistry, 47*, 2213–2217.

Häkkinen, S. H., & Törrönen, A. R. (2000). Content of flavonols and selected phenolic acids in strawberries and Vaccinium species: influence of cultivar, cultivation site and technique. *Food Research International, 33*, 517–524.

Häkkinen, S. H., Kärenlampi, S. O., Heinonen, I. M., Mykkänen, H. M., & Törrönen, A. R. (1999). Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. *Journal of Agricultural and Food Chemistry, 47*, 2274–2279.

Holcroft, D. M., & Kader, A. A. (1999). Controlled atmosphere-induced changes in pH and organic acid metabolism may affect color of stored strawberry fruit. *Postharvest Biology and Technology, 17*, 19–32.

Kalt, W., Forney, C. H., Martin, A., & Prior, R. L. (1999). Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *Journal of Agricultural and Food Chemistry, 47*, 4638–4644.

Lee, K. S., & Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology, 20*, 207–220.

Manning, K. (1996). Soft fruits. In G. B. Seymour, J. E. Taylor, & G. A. Tucker (Eds.), *Biochemistry of fruit ripening* (pp. 347–373). London: Chapman & Hall.

Marco, G. S. (1968). A rapid method for evaluation of antioxidants. *Journal of American Oil Chemistry Society, 45*, 594–598.

Parr, A. J., & Bolwell, G. P. (2000). Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science of Food Agriculture, 80*, 985–1012.

Pelayo, C., Ebeler, S. E., & Kader, A. A. (2003). Postharvest life and flavor quality of three strawberry cultivars kept at 5 °C in air or air + 20 kPa CO_2. *Postharvest Biology and Technology, 27*, 171–183.

Price, K. R., Prosser, T., Richetin, A. M. F., & Rhodes, M. J. C. (1999). A comparison of the flavonol content and composition in dessert, cooking and cider-making apples; distribution within the fruit and effect of juicing. *Food Chemistry, 66*, 489–494.

Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., & Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry, 66*, 401–436.

Swain, T., & Hillis, W. E. (1959). The phenolic constituents of Prunus domestica. I. The quantitative analysis of phenolic constituents. *Journal of the Science of Food Agriculture, 10*, 63–68.

Tomás-Barberán, F., & Clifford, M. N. (2000). Dietary hydroxybenzoic acid derivatives – nature, occurrence and dietary burden. *Journal of the Science of Food Agriculture, 80*, 1024–1032.

van der Sluis, A. A., Dekker, M., de Jager, A., & Jongen, W. M. F. (2001). Activity and concentration of polyphenolic antioxidants in apple: effect of cultivar, harvest year, and storage conditions. *Journal of Agricultural and Food Chemistry, 49*, 3606–3613.

Wang, H., Cao, G., & Prior, R. L. (1996). Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry, 44*, 701–705.

Watson, R., Wright, C. J., McBurney, T., Taylor, A. J., & Linforth, R. S. T. (2002). Influence of harvest date and light integral on the development of strawberry flavour compounds. *Journal of Experimental Botany, 53*, 2121–2129.

Wills, R. B. H., Wimalasiri, P., & Greenfield, H. (1984). Dehydroascorbic acid levels in fresh fruit and vegetables in relation to total vitamin C activity. *Journal of Agricultural and Food Chemistry, 32*, 836–838.

Zafrilla, P., Ferreres, F., & Tomas-Barberán, F. A. (2001). Effect of processing and storage on the antioxidant ellagic acid derivatives and flavonoids of red raspberry (*Rubus idaeus*) jams. *Journal of Agricultural and Food Chemistry, 49*, 3651–3655.