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Physico-chemical changes related to quality of five strawberry fruit cultivars during cool-storage

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Abstract

Several parameters related to strawberry quality, such as texture, anthocyanin content, titratable acidity, pH, total ascorbic acid, and total soluble sugars, were evaluated over a week of cool storage. The results indicated that low temperature used to increase strawberry shelf-life could also induce small changes in some of the quality parameters studied. However, the data revealed the importance of the cultivar in question since there were some contrasting responses among the cultivars. Also the initial values for some of the parameters were clearly different, indicating that the cultivar is the most important factor for determining post-harvest quality and extended shelf-life. Texture is an important parameter with regard to shelf-life but was not determined since the 'Mazi' cultivar had a high initial value for texture but no changes occurred over the storage period. However, it had the shortest shelf-life. Changes in anthocyanin content were highly dependent on the cultivar. Except for 'Toyonoka,' no marked changes were observed in titratable acids or pH. Ascorbic acid decreased by 50% in all cultivars. The profiles of soluble sugars were different among cultivars but, for all of them, sucrose disappeared on day 2 of storage.

Keywords: Strawberry; Cool storage; Anthocyanin; Ascorbic acid; Soluble sugars; Texture

1. Introduction

Strawberry is a non-climacteric fruit and it must be harvested at full maturity to achieve the maximum quality in relation to flavour and colour. The main changes in fruit composition which are usually associated with ripening, take place when the fruit is still attached to the mother plant. As a consequence, strawberries should be harvested ready for consumption. This means there is a very short period of fruit at its best quality. Besides mechanical injury, the other cause of strawberry spoilage after harvest is mold growth. Since the use of fungicide is not allowed, low temperatures and modified atmospheres are the tools for avoiding, at least partially, mold growth and fruit senescence, and thus extending strawberry shelf-life (Manning, 1996).

In spite of the wide use of low temperatures in strawberry preservation, not much is known about the effects of cool storage on chemical composition, in particular compounds with nutritional value (Montero, Mollá, Esteban, & López-Andréu, 1996). When strawberry fruits are stored at low temperature, their shelf-life can be extended to at least one week. Nevertheless, the delay between harvest and storage at the proper temperature is critical for the success of the treatment. It was observed that fruits stored at low temperature, 6 h after harvest, showed undesirable changes in colour and texture and also a reduction of around 50% of water content in comparison to those that were immediately cooled after harvest (Nunes, Brecht, Morais, & Sargent, 1995).

In the recent past, flavour and appearance were the most important attributes of fruits and other fresh vegetables, but nowadays consumers are more concerned about food safety and nutritional value. This is clear when one considers the increasing preference for organic products and fruits rich in nutrients and other beneficial compounds. The main characteristics related to the quality of ripe strawberry fruit are texture, flavour (soluble sugars and organic acids) and colour

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(anthocyanin content). Change in texture is a consequence of the natural process of senescence and also of the atmosphere in which the fruit is stored. Besides the obvious changes in appearance, mold contamination can also promote undesirable changes in texture and contribute to reduced strawberry shelf-life.

The increasing demand for dietary compounds with antioxidant action has focussed interest on fruits as natural sources of these compounds. In this respect, strawberry is a good source of ascorbic acid (AA) and flavonoid compounds (Wang, Cao, & Prior, 1996). Since fruits are no longer only ''attractive foods'', more effort should be made to understand the effects of treatments to increase shelf-life and improve nutritional value. Several research works have aimed to find the best compromise between extended shelf-life and maintenance of nutritional value. Modified atmosphere, which can be produced by increasing CO_2 level while reducing O_2, has yielded good results regarding strawberry preservation. However, colour was negatively affected, probably as a consequence of the inhibition of enzymes related to anthocyanin synthesis; also, reduction in ascorbic acid content during storage was observed (Wills, Ku, & Leshem, 2000). Lowering of ascorbic acid content could be related to the activity of ascorbate oxidase, which promotes the oxidation of ascorbic acid to dehydroascorbic acid (Agar, Streif, & Bangerth, 1997). Mehlhorn (1990) detected an increase in the activity of ascorbate peroxidase after ethylene treatment. It is possible that high levels of CO_2 could reduce the amount of ascorbic acid by increasing the level of ethylene.

The objective of this work was to study how texture, chemical composition and nutritional value of five strawberry varieties are related during 1-week of cool storage. The temperature of 6 °C was used because it is the average temperature usually applied to keep the fruit, when available for the final consumers.

2. Material and methods

2.1. Material

Strawberries (*Fragaria ananassa* Duch.), of the cultivars 'Dover', 'Campineiro', 'Mazi', 'Toyonoka' and 'Oso Grande', were all obtained on the same day, on the day of harvest from a local producer in Atibaia (São Paulo State). Fruits were harvested at commercial maturity (25–30 days after anthesis), when they were at full size and at least half red, and submitted to cold storage within at least 4 h after harvesting. Temperature treatment consisted in keeping the freshly harvested fruit, in transparent polystyrene baskets with a capacity of 500 g of strawberries, in the same way that they are usually exposed in the supermarket displays, in a chamber kept at 6 °C and room humidity around 95%. Ten fruits of each cultivar were sampled for texture analysis each day. The halves of the fruits that were not used in the texture analysis were immediately frozen in liquid nitrogen and stored at −80 °C for analysis of soluble sugars, anthocyanin content, total ascorbic acid content, citric acid, pH and total soluble solids (°Brix). Sampling of the cool stored strawberries was limited by the natural senescence of the fruits.

2.2. Anthocyanin content

A strawberry sample (2 g) was ground with 20 ml of methanol in 1% HCl, using an a Brinkmann Homogenizer (Polytron®—Kinematica GmbH, Kriens-Luzern, Sweden), and centrifuged at 2000 *g* for 15 min. Anthocyanin content was estimated as pelargonidin 3-glucoside at 510 nm, using a molar absortivity coefficient of 36,000.

2.3. 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, Calif, USA), using a PA_1 column (Dionex, Sunnyvale, Calif, USA) in an isocratic run of 18 mM NaOH during 25 min. Total soluble sugars were taken as the sum of glucose, fructose and sucrose values.

2.4. Total ascorbic acid determination

Ascorbic acid (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 type (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 DHAA with 10 mM DTT.

2.5. Titratable acidity, texture and total soluble solids

Total soluble solids (TSS) expressed as Brix, were measured with an Abbe refractometer (Carl Zeiss Jena—Germany) calibrated against sucrose. Texture analyses were done using a texturometer, TA-X12, equipped with a TA-7 USDA Warner-Blatzler knife (SMS Godalming, Surrey, England) to determine the force of shearing pieces of fruit (1 cm) at a speed of 5 mm/s. The pieces were taken from the equatorial sections, avoiding the edges of the fruits. For each stage, 10 fruits were analyzed. Titratable acidity (TA) was measured according to AOAC method 942.15 (1995) and expressed as citric acid.

3. Results and discussion

3.1. Texture

The values of texture, measured on the first day of cold storage, ranged from 0.6 N for 'Campineiro' to more than 1.0 N for the 'Oso Grande' (Fig. 1). Besides the difference in initial values of the texture, the cultivars were also different in relation to changes suffered during cold storage. While 'Oso Grande' and 'Mazi' cultivars showed a quite stable firmness throughout the storage period, cultivars 'Dover', 'Toyonoka' and 'Campineiro' became softer. This change was more evident in strawberry 'Campineiro', which had a decrease in texture of around 30% by the end of the storage period. At day 6, this variety was very poor in appearance, as a consequence of the deteriorative changes associated with senescence. Dramatic changes in texture would shorten the shelf-life of the fruits since they would become prone to mechanical injury and to mold contamination. However, an unexpected result was that obtained for 'Mazi' cultivar which, in spite of the constant and high values of firmness, had a short shelf-life. At day 5 the fruits were in very bad condition as a consequence of the advanced senescence. This is the reason why sampling of 'Mazi' was finished one day before that for the other cultivars. The better characteristic texture of the fruits of 'Oso Grande', 'Dover' and 'Toyonoka' cultivars, associated with its constancy during storage, makes them the most suitable for 1-week of cool storage. These cultivars would also be an interesting choice when handling the fruit, since they are more resistant to mechanical injury or abrasion.

3.2. Anthocyanin

According to the literature, the biosynthetic pathway for anthocyanin is still operative after strawberry harvest, and storage at low temperatures does not inhibit this process (Holkroft & Kader, 1999; Kalt & Macdonald, 1996). However, low temperature, combined with modified atmosphere, produces an inverse relationship between CO_2 concentration and anthocyanin content (Gil, Holcroft, & Kader, 1997). Since modified atmosphere was not used in the experiment described here, the profile of anthocyanin content (Fig. 2) during the storage period can be attributed only to the low temperature. In this respect, the cultivar was the main variable behind the observed differences in anthocyanin content. Fig. 2 shows that, not only were the initial amounts of anthocyanins different between cultivars, but also the speed of changes associated with storage at low temperature depended on the cultivar studied. The initial content of anthocyanin was as high as 60 mg/100

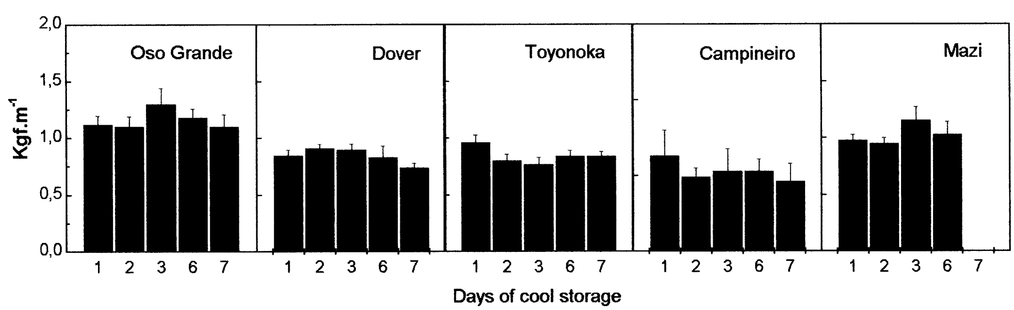

Fig. 1. Effect of cool storage at 6 °C on texture of five strawberry cultivars. The data presented are the means of 10 assays ± standard error after 1, 2, 3, 6 and 7 days of cool storage (except for Mazi cultivar).

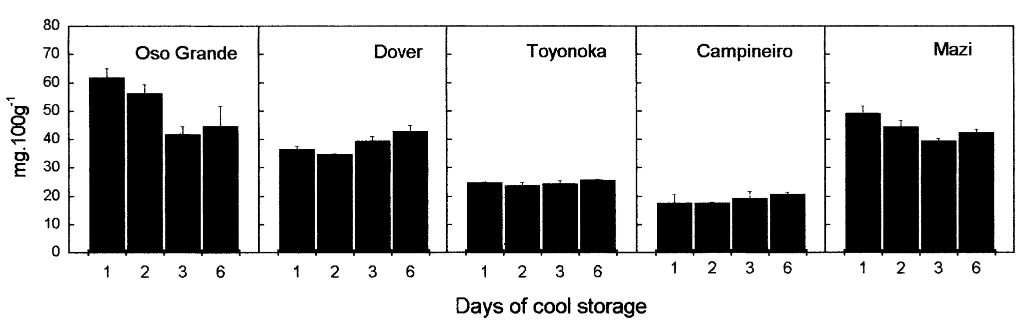

Fig. 2. Effect of cool storage at 6 °C on anthocyanin content of five strawberry cultivars. Data presented are the means of triplicate assays ± standard error after 1, 2, 3, 6 and 7 days of cool storage.

g in ‘Oso Grande’ cultivar and a little smaller in ‘Mazi’ and ‘Dover’, while the content of this pigment was significantly lower in ‘Toyonoka’ and ‘Campineiro’. In these cultivars, the anthocyanin content was below 30 mg/100 g. However, in spite of the low initial content, the amount of anthocyanin was almost constant during the period of cold storage. For ‘Campineiro’ and ‘Dover’ cultivars, the data obtained suggest a small increase (around 20%) in concentration of pigment. Those cultivars, initially rich in anthocyanin are in an opposite situation, since the content was clearly reduced after a 6-day storage at 6 °C. Anthocyanin contents of ‘Oso Grande’ and ‘Mazi’ were reduced by 27 and 14%, respectively. It seems possible that the contrasting trends observed in anthocyanin content changes are dependent on the initial amount but ‘Dover’, which can be considered as an anthocyanin-rich strawberry, showed an increase in its content during the cold storage.

Although changes in pigment content were detected, there were no associated visible changes in colour, which could affect the consumer’s choice. However, considering the beneficial effects brought by the reducing power of anthocyanin, a decrease in quality would be associated with the storage of strawberry of ‘Mazi’ and ‘Oso Grande’ cultivars. Since these varieties were also the richest in anthocyanin, this characteristic would have a compensatory effect.

3.3. Titratable acidity, pH and ascorbic acid

The percentages of titratable acidity (TA), in all cultivars studied were between 0.6 and 0.7 during the storage period (Fig. 3). These values are close to the lowest value mentioned in the literature, between 0.6 and 2.3% (Green, 1971; Montero et al., 1996). No marked changes in citric acid content were observed during fruit storage at cool temperature, except for ‘Toyonoka’ cultivar which showed an increase of around 20%. In agreement with the results obtained for organic acids, pH of all cultivars ranged between 3.6 and 4.1, values that were above the average for ripe strawberry, pH of 3.3 (Green, 1971). As was obtained for TA, no significant changes in pH were observed during storage of the fruits for 6 days at 6 °C. Again, the exception was ‘Toyonoka’ cultivar, which showed a decrease of pH from 4.1 to 3.8.

Organic, non-volatile acids are the second most important component of strawberry flavour, after soluble sugars. The main compound accounting for titratable acid (TA) is citric acid, which is predominant (over 90%) in strawberry. These acids regulate the cellular pH and may influence the anthocyanin stability and, as a consequence, the colour of the fruit. However, there is little published information about changes of pH and TA content in strawberry fruit stored at low temperatures. According to Nunes et al. (1995), cultivars ‘Chandler’, ‘Oso Grande’ and ‘Sweet Charlie’, stored at

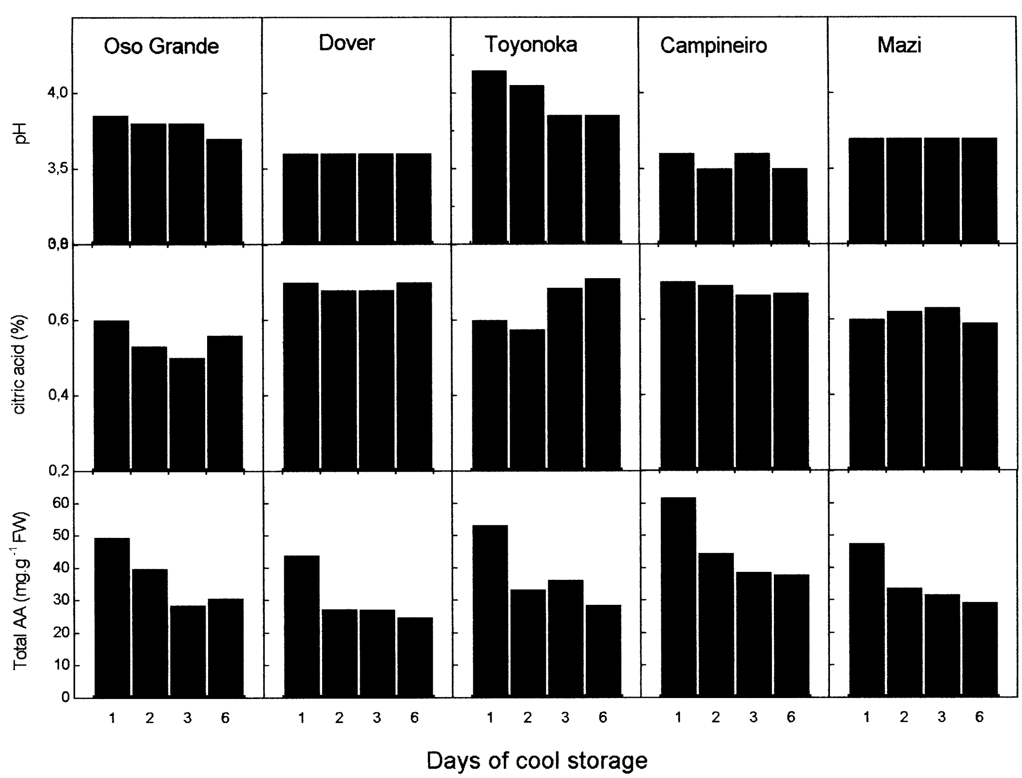

Fig. 3. Effect of cool storage at 6 °C on pH, citric acid (%), and total ascorbic acid (mg g^{-1} FW) contents of five strawberry cultivars. Data presented are the means of triplicate assays after 1, 2, 3, and 6 days of cool storage. The standard errors were below 5% in all cases.

1 °C, showed no differences in pH, but TA was slightly lower after 1 week. Remarkable changes of pH and citric acid content were observed by Gil et al. (1997) in stored strawberries of 'Selva' cultivar at 5 °C under different concentrations of CO_2 for 10 days. However, it was concluded that the effect was probably a consequence of the CO_2 since the increase of pH (and the decrease of TA) was paralleled by the increase in concentration of CO_2 in the atmosphere.

The results presented here (Fig. 3) clearly indicate that changes in pH and citric acid content during storage can also depend on the cultivar. This information is important if there is a correspondence between the observed change in composition and fruit taste, since organic acids are one of the main components of strawberry flavour.

In view of the importance of the strawberry fruit as a source of vitamin C in the human diet, the total ascorbic acid (AA) was analyzed in the cultivars during the cool storage period. The initial content of AA ranged between 62 and 44 mg/100 g of fresh fruit, in 'Campineiro' and 'Dover' cultivars, respectively (Fig. 3). In spite of the differences related to the variability among cultivars, the general responses of vitamin C losses during storage were the same, since the amounts of AA had decreased by about 50% in all cultivars at day 6. Temperature management after harvest is considered to be the most important factor in the maintenance of vitamin C content in fruits and vegetables. Except for some chilling-sensitive crops, the lower the temperature, the less the vitamin C losses (Lee & Kader, 2000). According to Nunes et al. (1995), a short delay before cooling is important for vitamin C maintenance in strawberry fruit. Ezell and Wilcox (1959) found that the temperature of storage and the rate of wilting in kale were determinants of losses as severe as 89% after 2 days at 20 °C. It is commonly assumed that low temperature has a protective effect on AA content in fruits and vegetables, and these results do not invalidate this consensus because of the 4-h delay between harvesting and cooling. A deleterious effect of room temperature, which could be as high as 30 °C at the moment of harvest, on AA stability cannot be ruled out. The homeostasis of AA may be impaired by a combinatory effect of stress, introduced by detachment from the mother-plant, and high temperature. In this way, strawberry fruit would be extremely prone to AA losses soon after harvest, which might account for the losses observed from day 1 to 2. Thus, minimum delay would slow down the oxidative degradation of the remaining AA after harvest.

3.4. Total soluble sugars

At day 1, the amount of total soluble sugars (TS-sugar) was below 40 mg/g for 'Campineiro' and 'Dover' cultivars, while 'Oso Grande', 'Toyonoka' and 'Mazi' had values above 50 mg/g. Table 1 shows that the TS-sugars were changed after 2 days of cold storage in all cultivars. Throughout the storage period the TS-sugar value was increased in all of them and, excepting for the 'Oso Grande' and 'Campineiro' fruits, the final sugar contents were similar or even higher than that at day 1. Since strawberry does not have starch to support soluble sugar synthesis after harvest, this increase may be a consequence of cell-wall degradation. This supposition accords with no changes in texture and no recovery of soluble sugar in 'Oso Grande' cultivar, but not in 'Campineiro' since it had the strongest changes in terms of texture, but this is not reflected in sugar content.

When total soluble solids (TSS) contents were analyzed, the results were similar to those observed for TS-sugar (Table 1). The initial values for TSS ranged from 6 to 9% on the first day of storage in all cultivars and changed in a similar way to total soluble sugars. This is an expected result since TSS are mainly related to soluble sugars in strawberry fruit. However, when one considers the sugar/acid ratio, 'Dover' and 'Campineiro' cultivars were close to the literature values throughout the storage time. Nevertheless, considering that an acceptable strawberry flavour is achieved with a minimum TSS of 7% and a maximum TA of 0.8% (Manning, 1996), they were not of the best quality for consumption. On the other hand, the sugar/acid ratios, for 'Oso Grande', 'Toyonoka' and 'Mazi' cultivars were much higher (Table 1) than the average values (5.3) quoted in the literature (Green, 1971) and TSS and TA were adequate to achieve the best quality.

As can be seen in Fig. 4 the main soluble sugars detected in strawberry were glucose, fructose and sucrose, showing the same proportions in 'Oso Grande' and 'Mazi' cultivars freshly harvested. Fructose was the

Table 1
Total soluble solids (TSS) (%), total soluble sugar (TS-sugar) (mg/g) and total soluble sugars/titratable acidity ratio (S/A) during cool storage of five strawberries cultivars

Days	Oso Grande			Toyonoka			Mazi			Dover			Campineiro		
	TSS	TS-sugar	S/A	TSS	TS-sugar	S/A	TSS	TS-sugar	S/A	TSS	TS-sugar	S/A	TSS	TS-sugar	S/A
1	8.5	58.8	9.9	9	52.6	8.9	7.4	52.7	8.8	6	37.3	5.5	6.2	38	5.4
2	7.5	44.2	8.3	10.2	68.9	12	7.6	35.5	5.7	5.5	34.4	5.1	6	29.3	4.2
3	6.5	44.4	8.9	9.5	40.8	6	7.6	47.1	7.5	6.4	31.5	4.6	6	28.3	4.2
6	7.1	47.2	8.4	9.6	61.3	8.6	8.9	62.9	10.7	6.4	41.0	5.8	6	38.7	5.8

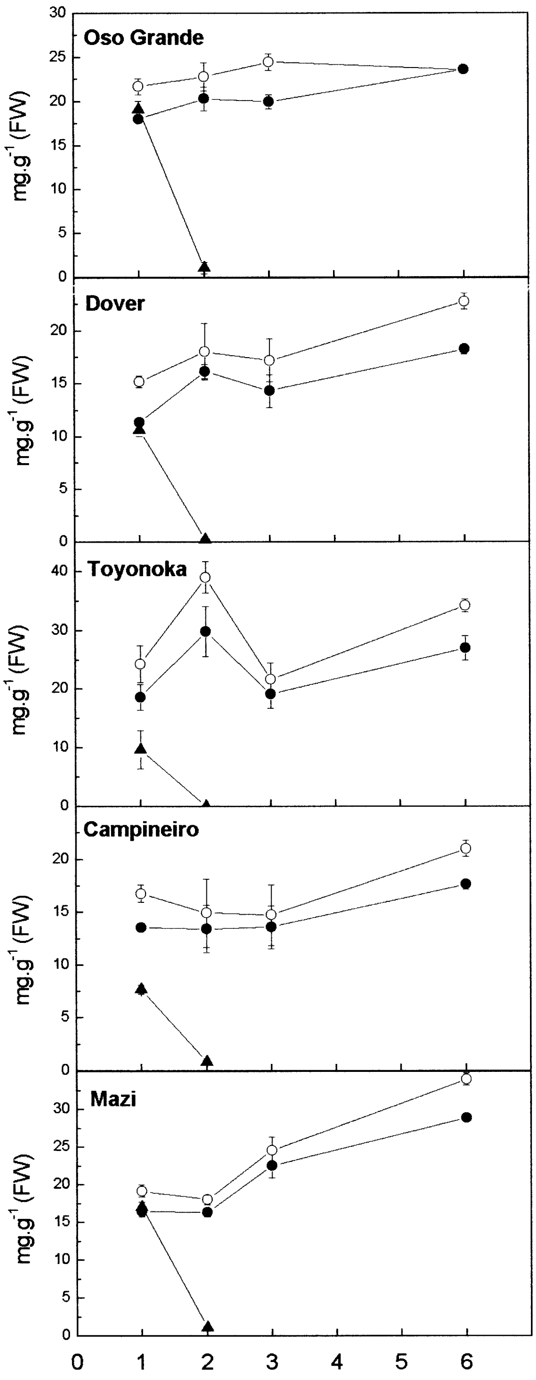

Fig. 4. Effect of cool storage at 6 °C on glucose (●), fructose (○) and sucrose (▲) contents of five strawberry cultivars. Data presented are the means of triplicate assays ± standard error after 1, 2, 3, and 6 days of cool storage.

major sugar (37–46%) in all cultivars, glucose being the second one. In spite of the minor differences observed in the total soluble sugar contents of 'Campineiro', 'Dover' and 'Oso Grande' cultivars harvested in the years 1999 (Cordenunsi, Nascimento, Genovese, & Lajalo, 2002) and 2000 (present data), the proportions between glucose, fructose and sucrose were almost the same. These results agree with the literature in that the content of individual sugars and total sugars varied with the station but the contents of individual sugars (as a percentage of the total sugar) did not vary with geographical position, temperature or cultivar (Manning, 1996).

As storage proceeded, sucrose decreased to undetectable levels while glucose and fructose showed a slight increase (Fig. 4). For 'Toyonoka' cultivar, the decrease in sucrose content was parallel to an increase of the amounts of other soluble sugars, which is an indication of high invertase activity. A similar situation can be proposed in the other cultivars, but a higher metabolic activity at the storage temperature could account for the consumption of sucrose by the tissue. This could explain why the disappearance of sucrose did not result in an increase in the contents of glucose and fructose. Forney and Breen (1986) found a sharp decrease of sucrose but only in an over-ripe strawberry. It is not known whether the disappearance of sucrose on day 2 was a consequence of the low storage temperature. Nothing is mentioned in literature about sucrose disappearance in freshly harvested strawberry.

4. Conclusions

The results indicated that low temperature, used to increase strawberry shelf-life, could also induce small changes in some of the quality parameters studied. However, the data obtained reveal the importance of the cultivar in question since there were some contrasting responses among the varieties. Also, the initial values for some of the parameters were clearly different, indicating that the cultivar of strawberry is the most important factor in determining post-harvest quality and extended shelf-life. This idea is reinforced by the data related to fruits cultivated at the same time under the same growing conditions. Another important point arising from the results is that cold storage is an efficient way to preserve strawberries, since no deleterious changes were observed either sensorially or nutritionally, at least considering the parameters above. The adverse effects observed by other authors may be related to the modified atmospheres employed in those studies.

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