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Growth and ripening season effects on antioxidant capacity of strawberry cultivar Selva

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Abstract

There is wide awareness on the importance of antioxidants in cell protection against free radicals constantly produced by the cell metabolism. In this work, carried out with strawberry cultivar ''Selva'', the antioxidant capacity and content of the main accepted antioxidants – ascorbic acid, dehydroascorbic acid, total phenols and anthocyanins – were determined along 10 growth stages of the fruit. Strawberries were harvested in winter and summer. Minimum/maximum temperatures in the two seasons were 6.3 °C/14.9 °C and 14.5 °C/27.8 °C, respectively. Antioxidant capacity in the small green stage was 27 mmol DPPH/100 g fresh tissue, keeping noticeably high values in the large-green one. Towards the white stage, antioxidant capacity reduced, to remain mostly constant. The antioxidant capacity was higher in those stages where cell division was more important and this parameter correlated mostly with the contents of total phenols and ascorbic acid.

Keywords: Strawberries; Antioxidants; Ascorbic acid; Anthocyanins; Phenols; Harvest season

1. Introduction

Fruits and vegetables are known lately to strongly contribute in reducing risks of diseases of various etiology as cancer and heart stroke. This fact is attributed to the large amounts of antioxidants they contain (Kris-Etherton et al., 2002; Häkkinen et al., 1999). Antioxidant compounds are produced by the plant to protect the cell against the attack from other cell chemical species as free radicals and reactive oxygen species. Free radicals are constantly produced by the cell metabolism (Benavente-García et al., 1997) and their concentration increases under stress situations (Nieman et al., 2002). They are responsible for lipid peroxidations, injury to plasmatic membrane, damage and rupture of proteins and deoxyribonucleic acid (Berlett and Stadtman, 1997). Antioxidants act by neutralizing free radical activity. The antioxidant capacity of a compound mainly depends on its high reactivity against free radicals and the stability of the intermediate species formed (antioxidant radicals) (Bors et al., 1990). The capacity to neutralize free radical activity is based on the properties of a group of enzymes (superoxide dismutase, catalase, peroxidase, etc.) and phenolic compounds of various chemical structures (e.g. catechins, flavonols, anthocyanins) and vitamins (C, E, and A) (Rapisarda et al., 1999; Fang et al., 2002).

The antioxidant capacity of several polyphenols was studied (Hagerman et al., 1998) as well as the antiradical efficiency of various compounds (Sanchez-Moreno et al., 1998). A good correlation has been found between antioxidant capacity and polyphenols content in diverse medicinal plants as artichokes (*Cynara scolymus* L.), *Gynkgo biloba* (Pietta et al., 1998), edible plants as lettuce (*Lactuca sativa* L.), onion (*Allium cepa* L.), celery (*Apium graveolens* L.) (Hertog et al., 1992) and in tea (*Camellia sinensis* L.) (Yokosawa et al., 1998).

Lee and Kader (2000) have pointed out that ascorbic acid content can be modified by several preharvest factors. Among them, light intensity increases sugar production and, as a consequence, may increase ascorbic acid synthesis. Temperature has been mentioned causing an inverse effect (Wang and Camp, 2000); higher temperatures in day and night lead to decreased synthesis of sugar and ascorbic acid.

On the other hand, several authors have investigated variations in antioxidant capacity induced in plants by abiotic stress conditions. Prior et al. (1998) have evaluated the influence of phenols and anthocyanins on the antioxidant

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capacity of industrial berry varieties in two ripening stages of harvest. They found increased antioxidant capacity, total phenols and anthocyanins content in riper fruit. Likewise, Wang and Lin (2000) have reached similar conclusions when studying the development of various cultivars of blackberry, raspberry and strawberry.

The objective of this work was to study the concentrations of anthocyanins, phenols, ascorbic acid, dehydroascorbic acid as well as the antioxidant capacity in ten development stages of strawberry cultivar Selva (characterized by weight, volume, surface color and sugar content) and the effect of harvest season (winter or summer) on these parameters.

2. Materials and methods

2.1. Plant material

Strawberry cultivar ''Selva'', grown in greenhouses in the La Plata horticultural belt (Province of Buenos Aires, Argentina), was utilized. Harvest months were June (average minimum and maximum temperatures of 6.3 and 14.9 °C, respectively; total solar radiation 58,419 W/m^2) and December (average minimum and maximum temperatures of 14.5 and 27.8 °C; total solar radiation 190,283 W/m^2). Fruits were collected (twice in each harvest season) in ten development degrees, selected by size and color: small green (SG), large green (LG), white (W), 5% red, 25% red, 50% red, 75% red, 100% red, 100% bright red (BR) and 100% purple red (PR). On arrival to the laboratory, damage-free fruits were selected. Twenty-five fruits from each ripening degree were pooled and then analyzed.

2.2. Characterization of the ripening stages

To characterize the ripening stages of the plant material utilized here, the following determinations were conducted.

2.2.1. Weight and volume

Weight of fruits was determined in an analytical balance while the volume, by liquid displacement.

2.2.2. Surface color

A Minolta CR300 Series (Osaka, Japan) chromameter was used by determining the following color coordinates: lightness (L^*), chromaticity a^* (red-green) and b^* (yellow-blue) in the CIE scale. The equipment was calibrated with a standard white plate ($Y = 93.2$, $x = 0.3133$, $y = 0.3192$). The hue angle, which provides information on the basic color tint, was calculated ($h^\circ = \tan^{-1} b^*/a^*$) together with the chroma, which denotes saturation ($C = (a^{*2} + b^{*2})^{1/2}$). Color readings were carried out on 25 fruits, on three zones of each one.

2.2.3. Sugar content

To this end, strawberries in each ripening degree and harvest season, were cut and frozen in liquid nitrogen, then crushed in a laboratory mill (Janke & Kunkel Ika Labortechnik A10, Staufen, Germany). Sugars were extracted from 1 g of the crushed sample using 5 mL of ethanol 96% and centrifuged at 10,000 × g for 10 min. Xylose, glucose, fructose and sucrose were identified and quantified by HPLC, using an Accubond Amino 5μ column and acetonitrile:water (75:25) as running solvent. A refraction index detector was utilized. In turn, for identification and quantification purposes, standards solutions of xylose, glucose, fructose and sucrose, each of 1.0 mg/mL, were utilized.

2.3. Determinations

2.3.1. Sample preparation

The samples utilized in the following determinations were prepared as described for sugar analysis.

2.3.2. Anthocyanins content

From 1 g of crushed material, anthocyanins were extracted by employing 5 mL of HCl (1%)–methanol solution and then spectrophotometrically determined at 520 nm (Beckman DU 650). Results were expressed as nmol of pelargonidin 3-glucoside/100 g fresh tissue (ε = 36,000 L/mol cm).

2.3.3. Total phenols

Total phenols were extracted from the crushed material using ethanol 96% (1:5, w/v) and determined at 760 nm, using the Folin Ciocalteau reagent (Singleton and Rossi, 1965). Total phenols concentration was expressed as mg of catechin/100 g fresh tissue. A calibration curve was utilized with catechin standard prepared in ethanol (0.1–0.6 mg/mL).

2.3.4. Concentrations of ascorbic (AA) and dehydroascorbic acid (DHA)

From samples of 1 g of crushed material, extraction was conducted using 5 mL citric acid 3% (w/v) and the extracts were then centrifuged at 10,000 × g for 10 min. The AA content was determined by HPLC (Wimalasiri and Wills, 1983). Concerning DHA, it was measured indirectly, by difference, determining AA concentration before and after addition of dithiotreitol (1 mg/mL extract) which reduces DHA to AA (Gökmen et al., 2000). A C18 Beckman column was employed, being acetonitrile:water (70:30) the eluent, containing $(NH_4)H_2PO_4$ 0.01 M, pH 4.3. The solvent flow rate was 2 mL/min. A UV detector Waters 450 was used at 254 nm. Results were expressed as mg AA/100 g fresh tissue.

2.3.5. Antioxidant capacity

Antioxidants were extracted from freshly harvested strawberries. Fruits were frozen in liquid nitrogen and crushed with ethanol 96% (w/w) (1:5, w/v) in a laboratory mill. The antioxidant capacity was determined by using the chromogenic radical DPPH (2,2-diphenyl-1-picryl hydrazyl) (Arnao, 2000). The amount of antioxidants required to reduce the initial DPPH concentration to a half (mean effective concentration, EC_{50}) was determined (Brand-Williams et al., 1995). Based on this value of EC_{50}, the quantity of DPPH needed to react with the total amount of antioxidants contained in the ethanolic extract was calculated. Final results were expressed as mmol DPPH/100 g fresh tissue.

2.4. Experimental design

A factorial design was used, based on the ripening degree and harvest season. All extracts and determinations were carried out at least in duplicate and the entire experiment was carried out twice, in two consecutive years. Since results coming from the 2 years were similar, data provided here correspond to one of the experiments. Results were analyzed by ANOVA and the means compared with the LSD test with a significance degree $\alpha = 0.05$.

3. Results and discussion

3.1. Growth and color evolution

Table 1 shows the weight, volume, hue angle and chroma along the various ripening stages considered here. Fruits increased their weight and size with the progress of ripening keeping the weight/volume ratio close to unity. At the first developmental stages, hue angle was observed to vary between 110° and 115°, indicating a greenish-yellow coloration. In turn, the chroma parameter was about 45, suggesting an intense color. The hue angle decreased with the progress of ripening, representing red color development. A non-linear correlation was found between weight and hue angle (grade 3 polynomial) ($r = 0.972$). No significant differences were found between hue values along the various ripening stages in the two harvest seasons. For summer harvest, hue angle correlated significantly ($p < 0.05$) with fructose, glucose, sucrose, and anthocyanin content ($r = -0.645$, -0.621, -0.706 and -0.841, respectively). In winter, better correlations were observed, being the coefficients $r = -0.893$, -0.896, -0.899 and -0.879 for fructose, glucose, sucrose, and anthocyanin content, respectively.

Chroma parameter values presented two maxima, corresponding to ripening stages SG and 100% red. In turn, by comparing results from the summer and winter harvests, Chroma did show significant differences ($p < 0.05$) from stage 50% R to PR, the winter harvest rendering fruits with more vivid color.

3.2. Sugars

Sugars found were xylose, sucrose, glucose and fructose. The total amount of sugars increased along all the ripening stages analyzed (data not shown). Literature data shows that several strawberry varieties had a similar behavior, as pointed out for cultivars Dover, Campineiro, Oso Grande and Toyonoka (Cordenunsi et al., 2002), as well as for cultivars Selena, Eros, Kent, Evita and Fern (Sturm et al., 2003) and cultivar Chandler (Montero et al., 1996). Conversely, other varieties showed relative constancy in total soluble sugars, from fruits light green or white at the top to fruits completely ripe (e.g. cultivars Marmolada, Elsanta, Pegasus and Northaester) (Sturm et al., 2003).

Trace levels of xylose were found over the 10 stages investigated (data not shown), which coincides with results published by Macías-Rodriguez et al. (2002) and Sturm et al. (2003).

Sucrose levels (Table 2), initially very low, rapidly increased up to the end of ripening. Fruits harvested in June (winter) exhibited a continuous increase of sucrose up to 100% red. This increase was notorious at the first developmental stages in fruits harvested in December (summer), although final levels remain constant from 50% R stage on. Significant differences ($p < 0.05$) were found between harvests, being the level corresponding to PR stage in winter four times higher than the one in summer. Montero et al. (1996) have found a sucrose increase during development in strawberry cv. Chandler and then a decrease, as the fruit was totally ripe. Cordenunsi et al. (2002) have found an increase of sucrose while Sturm et al. (2003) encountered a decrease along the ripening process in most of the strawberry varieties investigated.

Glucose and fructose levels in stages SG and LG were higher than those of sucrose. Glucose and fructose contents (Table 2) experienced a significant increase ($p < 0.05$) during fruit development and their ratio kept close to unity in all stages. Concentrations of fructose and glucose, initially between 0.3 and 0.8 g/100 g fresh tissue have increased two to three-fold in the stage W. Results from December and June harvests were significantly different ($p < 0.05$), being the contents of the latter (when maximum and minimum temperatures were lower)

Table 1
Characterization of strawberry ripening stages by fruit weight, volume and surface color

Ripening stage	Average weight (g)	Average volume (mL)	Chroma		Hue°	
			Summer	Winter	Summer	Winter
SG	0.80	1.00	44.65	45.83	115.3	110.86
LG	1.96	2.50	39.77	41.93	116.9	110.97
W	4.79	3.89	34.57	31.34	116.4	110.57
5% R	3.71	5.00	31.62	31.68	112.2	102.33
25% R	5.58	6.25	31.77	31.66	99.46	91.17
50% R	5.79	6.50	32.77	37.94	93.07	74.37
75% R	7.19	7.86	36.68	41.45	82.25	64.73
100% R	9.48	10.42	39.11	47.62	61.13	53.47
BR	9.37	10.00	37.02	44.64	50.13	46.8
PR	11.50	12.72	32.28	41.21	45.02	43.23
$LSD_{0.05}$	2.23	3.70	3.10		10.80	

Table 2
Characterization of strawberry ripening stages by sugar content

Ripening stage	Glucose (g/100 g fresh tissue)		Fructose (g/100 g fresh tissue)		Sucrose (g/100 g fresh tissue)	
	Summer	Winter	Summer	Winter	Summer	Winter
SG	0.41	0.95	0.36	0.85	0.03	0.50
LG	0.51	1.20	0.58	1.15	0.07	0.82
W	1.07	1.62	1.13	1.60	0.35	1.08
5% R	1.23	1.70	1.19	1.66	0.70	1.42
25% R	1.62	1.73	1.33	1.72	0.68	1.47
50% R	1.74	1.81	1.43	1.80	0.87	1.19
75% R	1.52	1.90	1.49	1.92	0.80	1.67
100% R	1.62	2.47	1.48	2.30	0.99	3.50
BR	1.38	2.30	1.35	2.38	0.78	3.35
PR	1.61	2.28	1.54	2.20	0.81	3.30
$LSD_{0.05}$	0.30		0.18		0.14	

much higher. In fruits ranging from 100% red to PR harvested in winter, sucrose levels were higher than those of glucose and fructose. However, the ratio of sucrose to glucose + fructose was always less than one, showing the prevalence of reducing sugars. Concerning the content of sugars in other than Selva cultivars, Sturm et al. (2003) have found predominance of glucose (2.0 g/100 g fresh tissue) and fructose (2.2 g/100 g fresh tissue) over sucrose (0.6 g/100 g fresh tissue) and that fructose and glucose exhibited an equivalent increase during development. These findings corresponds to the mean contents of sugars in 13 cultivars. Cordenunsi et al. (2002) have studied six strawberry varieties and, in four of them, fructose and glucose values increased considerably.

3.3. Anthocyanins

In quantitative terms, the most important anthocyanin of strawberry cv. "Selva" is pelargonidin 3-glucoside, though pelargonidin 3-rutinoside and cyanidin 3-glucoside are also present (Gil et al., 1997). Fig. 1 shows anthocyanin contents along the ripening stages. Anthocyanins were detected only from stage W onwards, keeping very low values up to the 50% red stage. From this stage on, concentration increased exponentially reaching at PR stage values 100 times higher than those for W. By studying strawberries cv. Allstar over seven stages, Wang and Lin (2000), also found that anthocyanins began to increase from the white stage (3 mg/100 g fresh tissue) up to 100% red (39 mg/100 g fresh tissue). Similar results were found by Montero et al. (1996) for cultivar Chandler.

Concerning anthocyanin content in both harvest seasons, there were no significant differences ($p < 0.05$) between summer and winter. Wang and Zheng (2001) have measured the effect of the day/night temperature ratio on anthocyanin content in ripe strawberries from Earliglow and Kent cultivars. They found that anthocyanin content increased as the ratio of maximum to minimum temperatures became higher.

Our findings in strawberry cultivar Selva showed that anthocyanin content correlated well with total sugars content in the winter harvest ($r = 0.826$; $p < 0.05$).

3.4. Total phenols

Total phenols content in fruits SG was of 650 mg catechin/100 g fresh tissue. These initial value is shown in Fig. 2 to decrease by 80% in the W stage, to remain almost constant from then until PR stage.

In other nine strawberry cultivars, Wang and Lin (2000) and Montero et al. (1996) have found a similar behavior. The last authors have observed total phenolics to decrease from initial

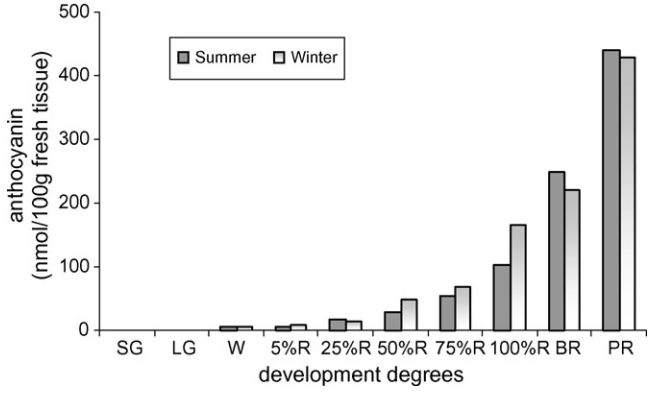

Fig. 1. Variation of anthocyanin content over the ripening stages of strawberry fruits, according to harvest season. $LSD_{0.05} = 71.4$.

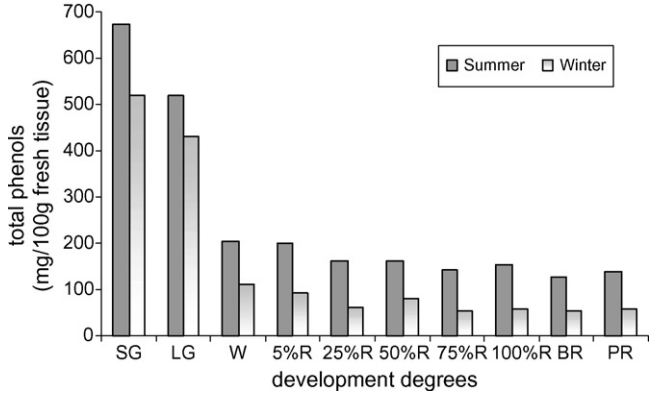

Fig. 2. Variation in the amount of total phenols during strawberry development and according to harvest season. $LSD_{0.05} = 21.07$.

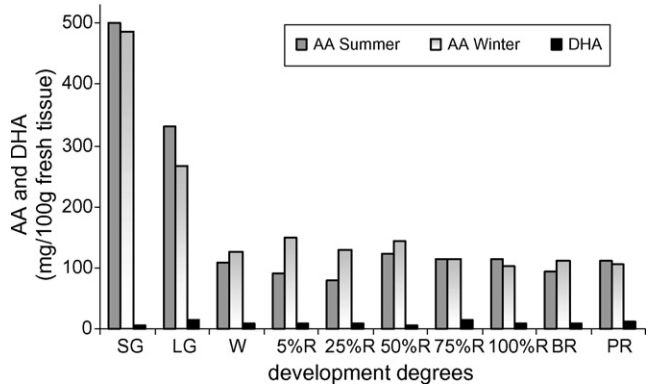

Fig. 3. Concentration of ascorbic acid (AA) – according to harvest season – and dehydroascorbic acid (DHA) along growth stages of strawberry. $LSD_{0.05}$ AA = 15.21.

1200 to 200 mg/100 g fresh tissue midway during fruit development.

Comparison of phenolics between the two harvest seasons led to significant differences ($p < 0.05$) being higher in the summer harvest. However, the variation along the ripening stages was similar in fruits harvested in the two seasons. Wang and Zheng (2001) and Ayala-Zavala et al. (2004) have also observed increased phenolics concentrations when growing fruits were exposed to higher temperatures.

In strawberry cultivar Selva, we have found that total phenols content correlated linearly with total sugars and fructose, glucose and sucrose, both in summer and winter ($r = -0.961$ and -0.785, respectively).

3.5. Ascorbic and dehydroascorbic acid

As observed in Fig. 3, the AA content was considerably higher than that of DHA, in agreement with reports by Agar et al. (1997) for cultivar Elvira. Concerning the evolution during the ripening stages of strawberry cv. Selva, the DHA concentrations were very low and almost constant while the AA level was, as mentioned above, always higher, in all the stages analyzed. No significant differences ($p > 0.05$) were found between the DHA data measured in the two harvest seasons.

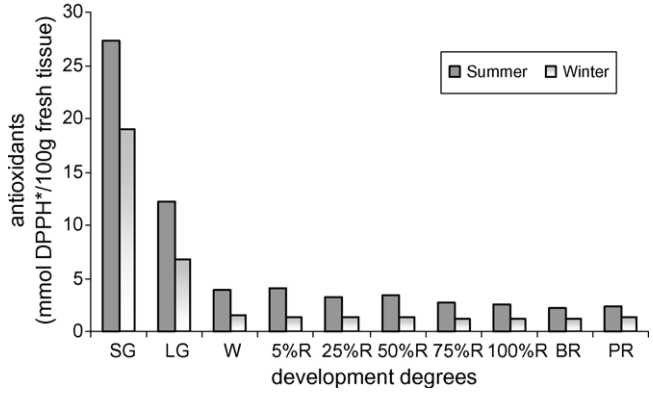

Fig. 4. Variation of antioxidant content during strawberry development, according to harvest season. $LSD_{0.05}$ = 1.43.

Over the first two development stages (SG and LG), very high AA concentrations were measured, possibly because of a higher proportion of interfering substances. From stage W onwards, AA level kept largely unaltered, at about 130 mg/100 g fresh tissue. In the cultivars utilized by Cordenunsi et al. (2002), increases in AA level were found during development, which ranged from 40 to 85 mg/100 g fresh tissue. Likewise, Montero et al. (1996) have observed a rise in AA along all the stages investigated, reaching 100 mg/100 g fresh tissue 35 days after fruit set. No significant differences ($p > 0.05$) were found between the data measured in the two harvest seasons.

In strawberry cultivar Selva, we have found that ascorbic acid content correlated linearly with total sugars, both in summer and winter ($r = -0.913$ and -0.769, respectively).

3.6. Antioxidant capacity

Fig. 4 shows a strong decrease in the antioxidant capacity of strawberry cv. Selva as fruits progress from stages SG to LG, where cell division and metabolism were more important, up to stage W. The antioxidant amount in stage SG was 27 mmol $DPPH^*$/100 g fresh tissue, a value that reduced by 90% when reaching the stage W (with respect to the initial value) to remain rather unchanged up to the end of fruit development. The summer harvest produced higher values of antioxidant capacity, showing a similar evolution to that exhibited by phenolic compounds.

Wang and Lin (2000), using the ORAC method, have observed the amount of antioxidants in stage SG to reduce to a half in stage W, to then keep mostly constant until the end of ripening.

As far as strawberry growth is concerned, Wang and Zheng (2001) have found the antioxidant power to increase for longer exposure of the plants to higher temperatures. This may be caused by the high antioxidant power of the phenolics, whose concentration also increases.

With regard to our studies, while the antioxidants amount decreased during fruit development, the anthocyanins increased by about 100 times.

The antioxidant capacity presented a linear correlation with the phenolics content ($r = 0.942$) and AA concentration ($r = 0.950$) but not with the concentration of anthocyanins. Therefore, the variation of antioxidant capacity followed the variations of phenolics and AA, but not the variations of anthocyanins concentration. Meyers et al. (2003) reported similar results in cultivars Earliglow, Annapolis, Allstar, Sable, Sparkle, Jewel and Messabi.

4. Conclusions

Since the offer of strawberry cultivars in the world market is highly dynamic, to know as much as possible information about quality characteristics for each cultivar, including nutritional composition, becomes a useful strategy. This knowledge may contribute to evaluate and select the most appropriate crop practices and to assign the best alternative of use (fresh market, industrial processing, etc.) to each material.

The results obtained in this research carried out with strawberry cultivar Selva suggested that:

- During fruit evolution, there were no significant differences in surface color, nor in the anthocyanins content between the fruits harvested in summer or winter.
- All sugars increased during ripening but the increase in sucrose was significant until the 100% red stage. Higher sugar contents were found in fruits harvested in winter.
- The AA content was not significantly affected by temperature during the growing season.
- The antioxidant content was much higher in stages SG and LG. Antioxidant capacity would correlate mostly with the evolution of total phenolics and the concentration of ascorbic acid, but not with that of anthocyanins. Concentrations of antioxidants and phenols were higher in fruits harvested in summer.

As a general conclusion, the winter harvest yielded fruits with a high quality from a nutritional point of view. This fact seems to be important since, using ''neutral to day'' cultivars, the production of strawberries could be concentrated in two periods of the year without apparent loss of product quality. Future needs for research would be related to assess if it is possible to maintain high levels of antioxidant compounds in ripe fruits since, at commercial maturity, antioxidant activity decreased notoriously.

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