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Antioxidant Activity and Sensory Changes of Strawberry Tree Fruits during Cold Storage and Shelf Life

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Abstract

Fadda C., Fenu P.A.M., Usai G., Del Caro A., Diez Y.M., Sanguinetti A.M., Piga A. (2015): **Antioxidant activity and sensory changes of strawberry tree fruits during cold storage and shelf life**. Czech J. Food Sci., 33: 531–536.

The influence of storage time on a range of quality parameters, *in vitro* antioxidant activity, and the sensory acceptability of fully ripe strawberry tree fruits packaged into polypropylene trays under plastic film was assessed. Fruits were stored at 0°C for 6, 12 or 18 days and then transferred to shelf life at 20°C for 2 days. The parameters most influenced by cold storage and shelf life were firmness, colour, and antioxidant activity. Firmness decreased significantly during storage and colour parameters showed a decrease in saturation. Significant increases in polyphenols and anthocyanins were registered during storage, with a resulting increase in total antioxidant activity. The sensory analysis demonstrated that fruits maintain an acceptable quality level for up to 1 day under shelf life conditions following 18 days of cold storage.

Keywords*:* anthocyanins, polyphenols, sensory analysis, texture

The consumption of fruits and vegetables is considered to be one of the most powerful ways to counterbalance the production of reactive oxygen species that are potentially mutagenic and oncogenic (Bouayed & Bohn 2010).

Berries are very rich in health-promoting phytochemicals (Fortalezas *et al*. 2010). An increasing interest has recently been directed towards the potential health-promoting capacity of the strawberry tree fruits (*Arbutus unedo* L., *Ericaceae*), which are mainly used to obtain distillates, jams, preserves, and jellies (Alarcao-E-Silva *et al*. 2001), while their consumption as fresh fruit is scarce. Guerreiro *et al*. (2013) reported that fresh strawberry tree fruits keep their nutritional and chemical quality for 15 days of storage at 0°C. However, to the best of our knowledge, no studies have yet investigated the influence of storage at shelf life conditions (SL), following cold storage (CS), upon changes in strawberry tree fruit quality.

With this in mind, we aimed to investigate changes in quality parameters and the *in vitro* antioxidant activity of wild strawberry tree fruits subjected both to CS and SL.

MATERIAL AND METHODS

Fruit selection, packaging and storage. Wild strawberry tree fruits were harvested from a wood located in Alghero, NW Sardinia (Italy), and transported to the laboratory inside a refrigerated box. Two thousand four hundred fully ripe fruits free of defects and with a diameter ranging from 1.5 cm to 2 cm were selected, placed in 350 ml polypropylene trays (15 fruits per each tray), which were then hand wrapped using a 9 mm polyvinylchloride film and transferred to 0°C and 90–95% relative humidity (RH) for 18 days. Each tray was weighed prior to storage to calculate weight loss. After 6, 12, or 18 days of CS, 30 trays were transferred to SL at 20°C and 70% RH and changes in fruit properties were assessed following 1 and 2 days of these SL. A maximum of just 2 days of SL was considered since

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the fruits are very perishable. For each sampling time, 16 trays were considered, of which 10 were used for sensory analyses. The content of the remaining 6 trays was used in groups of 3 replications of 10 fruits for the other analyses.

General quality parameters: SSC, pH, titratable acidity, colour, texture analysis, weight loss and mould incidence. The fruits were homogenised and subjected to the analysis of pH, titratable acidity (Tac), % of soluble solids content (SSC), and dry matter (DM %), by using routine methods.

Skin **c**olour was measured with a tristimulus colorimeter (Chromameter-2 Reflectance; Minolta, Osaka, Japan) equipped with a CR-300 measuring head. The L^*, a^*, and b^* CIELAB parameters were used and hue angle ($\tan^{-1} b^*/a^*$) and Chroma $(a^2 + b^2)^{0.5}$ were computed.

Changes in texture over time were measured with a texture analyser (TA.XT plus; Stable Microsystems, Surrey, UK). A puncture test and Texture Profile Analysis (TPA) were performed using 15 fruits for each determination. The puncturing was performed on the fruit lateral face with a 3 mm diameter (SMS P/3) needle probe. Three indexes were considered: the fruit break force (yield point), as the maximum force (N) reached during the test; the hardness of the flesh, as the area (N/s) calculated between the starting point and the maximum force; and the gradient of stiffness (N/s) calculated between the start point and the yield point. The TPA was carried out on the lateral face of whole fruits in compression mode using a SMSP/75 plate. Hardness, cohesiveness, chewiness, and springiness were evaluated and calculated by the instrument's software.

Weight loss (%) was calculated on 5 trays, while mould incidence was expressed as % of rotten fruit.

Chemical analysis. Ascorbic acid (AA) was extracted as described by Serçe *et al.* (2011) and analysed (Orazek *et al.* 2011) using a HPLC System (110 Series; Agilent Technologies, Palo Alto, USA). The peak for AA was identified and quantified using external standards.

Total polyphenols (TP) were extracted as reported by Oliveira *et al.* (2011). Extracts were then subjected to spectrophotometric determination at 750 nm (mod. 8453; Hewlett-Packard, Palo Alto, USA).

Total anthocyanins (TANT) were extracted according to Alarcao-e-Silva *et al.* (2001), and determined spectrophotometrically using a pH differential method (Lee *et al.* 2005). Concentration was expressed as cyanidin-3-glucoside equivalents (Guerreiro *et al.* 2013).

Antioxidant activity (AC) determination. Antioxidant activity was evaluated using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). A diluted and filtrated sample obtained as reported by Piga *et al*. (2003) was used and the antioxidant activity was expressed with the following – $OD^{-3}min^{-1}g^{-1}$ DM, in the Equation:

$$(1/A^3) - (1/A_0^3) = 3kt$$

where: A_0 – initial optical density; A – optical density at rising time t; OD – optical density

Sensory analysis. A laboratory test was used to evaluate sensory changes after different storage times. Specifically, 25 untrained consumers were asked to evaluate the acceptability of the samples using a hedonic scale ranging from 1 to 7 (1 – dislike extremely; 4 – acceptable; 7 – like extremely) for the following attributes: appearance, acidity, sweetness, flavour and firmness. An overall acceptability score was also computed as the mean of the 5 attributes. A score of 4 was used as the threshold for product acceptance.

Statistical analysis. All determinations were performed in triplicate unless otherwise indicated. Data were evaluated by one-way analysis of variance (ANOVA) using Statistica 6.0 for Windows. Storage period was selected as a variable. Means, where appropriate, were separated by Tukey's test for $P \leq$ 0.05, 0.01 and 0.001.

RESULTS AND DISCUSSION

General quality parameters: SSC, pH, titratable acidity, colour, texture analysis, weight loss and mould incidence. The pH value dropped significantly after 6 days of CS and rose again back to initial values by day 12 of CS (Table 1). However, all values were always lower with respect to those for fresh fruits. SL did not result, in general, in changes of pH with respect to their corresponding CS. A similar, but opposite, trend was registered for Tac, which increased significantly compared to fresh fruit values at the end of the first 6-day CS period, and then remained relatively constant, with few exceptions. The pH and Tac data for fresh fruits are similar to those reported by other authors (Ganhão *et al.* 2010; Ruiz-Rodriguez *et al.* 2011), but the evolution of these parameters during CS and SL is reported here for the first time. The AA values are also similar to those reported in the literature (Alarcão-E-Silva *et al.* 2001; Orak *et al.* 2011) and did not change significantly during CS or SL, as already reported (Guerreiro *et al.* 2013).

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Table 1. Influence of storage time on some chemical and physical parameters, ascorbic acid content and weight loss of strawberry tree fruits

Storage time (days)[x]	pH	Titratable acidity (g malic acid/100 g DM)	Total soluble solids (°Brix)	Ascorbic acid (mg/100 g DM)	Weight loss (%)
0	3.68[a]	1.95[d]	22.65[d]	534[a]	0.00[g]
6	3.48[cd]	2.26[bc]	25.10[b]	480[a]	1.65[f]
6+1	3.55[b]	2.49[bc]	25.55[ab]	501[a]	2.22[de]
6+2	3.41[ef]	2.47[ab]	25.15[b]	666[a]	2.41[cd]
12	3.54[b]	2.39[bc]	25.05[b]	644[a]	2.09[e]
12+1	3.53[b]	2.18[c]	23.30[c]	668[a]	2.57[bc]
12+2	3.56[b]	2.49[a]	22.70[d]	718[a]	2.71[ab]
18	3.45[d]	2.27[bc]	25.85[a]	568[a]	2.23[de]
18+1	3.48[cd]	2.43[abc]	23.75[c]	663[a]	2.68[ab]
18+2	3.47[d]	2.34[bc]	22.55[d]	553[a]	2.92[a]
Significance	**	***	***	ns	***

[x]0, 6, 12, and 18 are CS days, while 6+1, 6+2, 12+1, 12+2, 18+1, and 18+2 are days of SL; data followed by different letters within each column are significantly different by Tukey's test; significance $^{**}P < 0.01$, $^{***}P < 0.05$; ns – not significan

Skin colour determination revealed a decrease in L^*, a^*, and b^* (Table 2). The decrease in L^* was significant only after 1 day of SL following 12 days of CS and was probably caused by enzymatic browning (Guerreiro *et al.* 2013). The L^* values of fresh fruits were similar to those reported by Orak *et al.* (2011). Both a^* and b^* decreased significantly during storage. Changes in the colour were only due to a decrease in saturation, as was also demonstrated by the concomitant decrease in the Chroma value, and the lack of any significant shift in the hue.

The puncture test revealed a significant decrease in fruit firmness during both CS and SL (Table 3). A dramatic loss of firmness is observed after 6 days of CS, after that changes were more limited, in accordance with data reported by Guerreiro *et al.* (2013) and with the results reported for other berry-like fruits (Cordenunsi *et al.* 2003).

The hardness and chewiness measured by TPA changed significantly after 6 or 12 days of CS, respectively, with respect to pre-storage values. Decrease in hardness is a clear sign of the flesh softening, thus confirming the results of the puncture test. The same trend was noticed for chewiness, while cohesiveness changed only after 12 days of CS. To the best of our knowledge, these are the very first

Table 2. Influence of storage time on the evolution of colour parameters of strawberry tree fruits

Storage time (days)[x]	L^*	a^*	b^*	Hue ($\tan^{-1}b^*/a^*$)	Chroma $(a^2 + b^2)^{0.5}$
0	35.61[a]	39.21[a]	38.12[ab]	45.74[ab]	54.74[a]
6	36.65[a]	38.31[a]	36.18[abc]	46.72[ab]	52.88[ab]
6+1	32.56[abc]	34.13[ab]	31.17[cbd]	47.72[ab]	46.30[bcd]
6+2	33.51[abc]	36.50[ab]	38.29[a]	43.77[b]	53.06[ab]
12	33.73[ab]	36.39[ab]	33.98[abc]	47.05[ab]	49.84[ab]
12+1	30.19[bcd]	36.29[ab]	29.52[cde]	50.93[ab]	46.84[abc]
12+2	29.22[bcd]	35.56[ab]	31.01[cd]	48.91[ab]	47.30[ab]
18	30.15[bcd]	33.88[ab]	29.83[cde]	48.48[ab]	45.20[bcd]
18+1	27.01[d]	30.88[b]	22.96[e]	53.44[a]	38.59[d]
18+2	28.61[cd]	30.05[b]	24.74[d]	50.22[ab]	39.09[d]
Significance	***	**	***	**	***

[x]0, 6, 12, and 18 are CS days, while 6+1, 6+2, 12+1, 12+2, 18+1, and 18+2 are days of SL; data followed by different letters within each column are significantly different by Tukey's test; significance $^{**}P < 0.01$, $^{***}P < 0.05$

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Table 3. Influence of storage time on the evolution of texture parameters of strawberry tree fruits

Storage time (days)^x	Texture test						
	TPA				Puncture		
	hardness (N)	springiness	chewiness (N/s)	cohesiveness	maximum force (N)	area (N/s)	gradient (N/s)
0	5.34^b	0.527^a	1.021^b	0.361^{ab}	1.328^a	0.919^a	1.346
6	6.95^a	0.535^a	1.047^a	0.385^a	0.672^b	0.311^{bc}	1.507
6+1	2.35^{cd}	0.483^{abc}	0.408^c	0.362^{ab}	0.688^b	0.158^c	0.626
6+2	2.83^{cd}	0.472^{abc}	0.439^c	0.324^{bc}	0.580^b	0.167^c	0.756
12	3.21^c	0.500^{ab}	0.558^c	0.347^{abc}	0.482^{bc}	0.272^{bc}	0.490
12+1	1.99^{cd}	0.450^{bc}	0.265^c	0.295^c	0.283^c	0.370^{bc}	0.402
12+2	2.32^{cd}	0.462^{abc}	0.320^c	0.296^c	0.286^c	0.337^{bc}	0.424
18	2.36^{cd}	0.537^a	0.493^c	0.390^a	0.470^{bc}	0.369^{bc}	0.634
18+1	1.62^d	0.413^c	0.202^c	0.302^c	0.505^{bc}	0.584^{ab}	0.708
18+2	1.45^d	0.464^{abc}	0.215^c	0.309^c	0.497^{bc}	0.303^{bc}	0.515
Significance	***	*	***	**	***	*	***

^{x}0, 6, 12, and 18 are CS days, while 6+1, 6+2, 12+1, 12+2, 18+1 and 18+2 are days of SL; data followed by different letters within each column are significantly different by Tukey's test; significance *P < 0.001, **P < 0.01, ***P < 0.05

data reporting the texture evolution using the TPA test during CS and SL of strawberry tree fruits.

Weight loss peaked at 3% by the end of the trial period, in agreement with Guerreiro *et al.* (2013), thus the packaging prevented the shrivelling of fruits. Weight loss during SL was significantly greater compared to that observed during cold storage. No moulds were detected throughout the whole trial.

Chemical analysis: AA, TP, TANT. The evolution of TP, TANT, and AC over the storage period is reported in Table 4. TP increased progressively and significantly up to six times during storage, with a sharp increase detected for the last two samplings (SL). We collected fruits at the stage of full ripeness as assessed by external colour, thus we would have expected to observe a slight decrease or constancy of values; it is probable that the process of overripening resulted in the increase of TP. Isbilir *et al.* (2012) found an increase in TP when fruits changed from the yellow to the red stage, but to the best of our

Table 4. Influence of storage time on total polyphenols, total anthocyanins and antioxidant activity of strawberry tree fruits

Storage time (days)^x	Total polyphenols (mg gallic acid/100 g DM)	Total anthocyanins (mg cyaniding/100 g DM)	Antioxidant activity (– OD^{-3}min^{-1}g^{-1} DM)
0	279.7^f	17.07^d	102.6^f
6	373.1^f	22.19^c	108.4^{ef}
6+1	570.4^e	24.56^c	118.7^{cde}
6+2	720.6^{de}	32.65^b	126.3^{bc}
12	755.4^{de}	24.80^c	118.8^{cde}
12+1	833.9^{cd}	30.58^b	114.4^{def}
12+2	957.5^c	42.34^a	125.6^{bed}
18	891.7^{cd}	41.87^a	107.5^{ef}
18+1	1182.1^b	42.51^a	137.4^{ab}
18+2	1392.5^a	45.99^a	143.5^a
Significance	***	***	***

^{x}0, 6, 12, and 18 are CS days, while 6+1, 6+2, 12+1, 12+2, 18+1, and 18+2 are days of SL; data followed by different letters within each column are significantly different by Tukey's test; significance ***P < 0.05

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Table 5. Influence of storage time on the evolution of sensory attributes of strawberry tree fruits

Storage time (days)^x	Appearance	Acidity	Sweetness	Flavour	Firmness
0^x	5.7^{ab}	4.7	5.8^{a}	5.0^{a}	5.7^{a}
6	5.7^{ab}	4.7	5.5^{ab}	4.8^{a}	5.3^{abc}
6+1	6.0^{a}	4.4	5.8^{a}	5.4^{a}	5.4^{ab}
6+2	5.6^{ab}	4.6	5.8^{a}	4.8^{a}	4.6^{bcd}
12	5.7^{ab}	4.5	5.7^{ab}	4.8^{a}	5.2^{abc}
12+1	5.0^{bc}	4.3	5.8^{a}	5.2^{a}	4.5^{cd}
12+2	4.6c	3.6	5.2^{ab}	4.4^{ab}	4.0^{de}
19	4.2^{c}	4.0	5.0^{ab}	4.4^{ab}	4.0^{de}
19+1	4.4^{c}	4.2	4.8^{b}	4.4^{ab}	4.0^{de}
19+2	4.2^{c}	3.3	4.8^{b}	3.7^{b}	3.3^{e}
Significance	***	ns	*	*	***

^{x}0, 6, 12, and 18 are CS days, while 6+1, 6+2, 12+1, 12+2, 18+1, and 18+2 are days of SL; data followed by different letters within each column are significantly different by Tukey's test; significance $^*P < 0.001$, $^{***}P < 0.05$; ns – not significant

knowledge, these constitute the first data on the evolution of TP during the storage of fresh strawberry tree fruits. Similarly, TANT showed a threefold increase between harvesting and the last sampling period, even with a slightly different pattern with respect to TP. A similar trend was recently observed by Guerreiro *et al.* (2013) for TANT.

Antioxidant activity. The increase in both TP and TANT resulted in a progressive and significant increase in the AC at the end of the storage period. To conclude, CS and SL both have a very beneficial effect upon increasing the health-promoting properties of these fruits.

Sensory analysis. The fresh fruits obtained an overall acceptability score of 5.38 that is between 'good' and 'very good' (Figure 1). The sensory attributes significantly changed during storage, however, with the exception of acidity, they remained above the chosen acceptance threshold until the 18+1 storage time (Table 5). Moreover, the attributes acidity, flavour, and firmness only dropped below the acceptability limit at the final sampling time (2 days of SL). Scores registered after the SL were not different from those obtained during the previous CS.

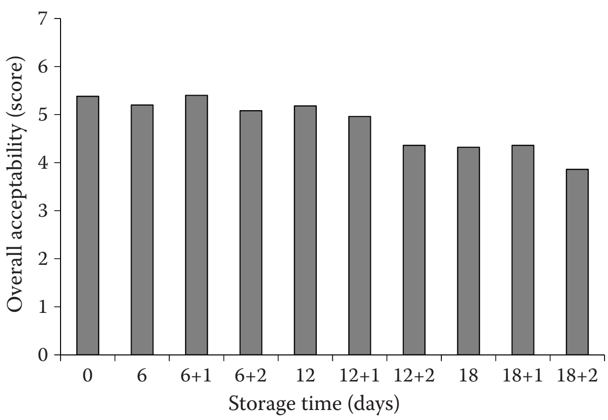

Figure 1. Change of overall acceptability scores during storage of fresh strawberry tree fruits

Changes in the sensory attributes resulted in a maximal 1.5 reduction in the overall acceptability score, which, nevertheless, always remained above the fixed acceptability threshold, with the only exception being the last sampling time (2 days of SL).

CONCLUSIONS

The results of this study reveal that strawberry tree fruits can withstand not only CS at 0°C but also a second day of SL at 20°C following 18 days of CS, as assessed by sensory analysis. They also maintain their good quality after 2 days of SL following 6 and 12 days of CS. However, fruits underwent overripening, as revealed by texture and colour evolution. Although this did not result in excessive impairment of their sensory acceptability, it did result in a significant increase in TP and TANT and in the related AC; thus storage at CS and SL actually improves their health-promoting properties. Moreover, the PVC wrapping helped maintain minimal weight loss and, combined with the low cold storage temperature, avoided the growth of moulds. It is to highlight, however, that due to the extreme fruit perishability, the fruits should be stored at temperatures lower than 4°C during the

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whole commercialization step, in order to extend their shelf life for a proper time.

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