FRUITS

ORIGINAL ARTICLE

Effect of the farming system and postharvest frozen storage on quality attributes of two strawberry cultivars

Giancarlo Barbieri, Emma Colonna, Youssef Rouphael⋆ and Stefania De Pascale

Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy

Abstract – Introduction. Consumer interest in organic fruit is increasing based on the assumption that these products are healthier, despite a lack of conclusive evidence. Strawberry (*Fragaria × ananassa* Duch.) is a rich source of antioxidants and an important fruit in the human diet. **Materials and methods**. The configuration of strawberry fruit quality was analyzed in a factorial approach accounting for the effects of the farming system and postharvest frozen storage. The strawberry cultivars 'Sabrina' and 'Ventana' grown under organic and conventional farming management were stored postharvest at −40 °C for 6 months. Fruits obtained under the two farming systems were analyzed fresh after harvest and thawed after frozen storage. Fruit quality was evaluated in terms of physical, chemical and phytochemical properties. **Results and discussion**. The surface lightness, firmness, lipophilic antioxidant activity, soluble solids, ascorbic acid and ellagic acid contents were significantly higher at harvest than after thawing. Frozen storage increased only hydrophilic antioxidant activity, leaving reducing sugars and total phenols without significant alterations. The color of organic fruits in both cultivars was brighter and more vivid (higher L* and C* values). Farming management did not affect the reducing sugars and ascorbic acid contents for the 'Sabrina' (4.6% and 55.5 mg 100 g^{-1} FW, respectively) or 'Ventana' (4.0% and 31.2 mg 100 g^{-1} FW, respectively) cultivars. The organic 'Ventana' strawberry had higher soluble solid content and hydrophilic antioxidant activity, whereas an opposite trend was observed in total phenols and ellagic acid contents for 'Ventana' and 'Sabrina', respectively. **Conclusion.** The quality of strawberries, in particular the bioactive compounds, varied according to the farming system and postharvest frozen storage. The cultivation factors, including the farming management and cultivar, may significantly interact to affect quality attributes of strawberry fruit.

Keywords: Italy / strawberry / *Fragaria × ananassa* / organic farming / fruit quality / antioxdant activity / ascorbic acid / ellagic acid / phenolics

Résumé – Effet du système de culture et de congélation post-récolte sur les attributs de qualité de deux cultivars de fraisier. Introduction. L'intérêt des consommateurs pour les fruits issus d'agriculture biologique repose de plus en plus sur l'hypothèse selon laquelle ces produits sont meilleurs pour la santé, malgré l'absence de preuves concluantes. La fraise (*Fragaria × ananassa* Duch.) est une source abondante d'antioxydants tout en étant un fruit courant en alimentation humaine. **Matériel et méthodes.** Les composantes de la qualité de la fraise ont été analysées selon une approche factorielle prenant en compte des effets du système de culture et de stockage post-récolte par congélation. Les fruits des cultivars de fraisiers 'Sabrina' et 'Ventana' cultivés en conduite soit biologique soit conventionnelle, ont été stockés à −40 °C après la récolte. Les fruits obtenus selon les deux systèmes de culture ont été analysés frais après la récolte et décongelés après 6 mois stockage. La qualité des fruits a été évaluée en termes de propriétés phytochimiques physiques, chimiques, etc. **Résultats et discussion.** La douceur de surface, la fermeté du fruit, l'activité anti-oxydante lipophile, les solides solubles, les teneurs en acide ascorbique et en acide ellagique étaient significativement plus élevées à la récolte qu'à la décongélation. La congélation a seulement contribué à augmenter l'activité anti-oxydante hydrophile, laissant les teneurs en sucres réducteurs et en phénols totaux sans modifications importantes. Dans le cas des deux cultivars, la couleur des fruits issus d'agriculture biologique était plus lumineuse et plus vive (valeurs de L* et de C* supérieures). La gestion biologique de la production n'a pas affecté les teneurs en sucres réducteurs ni en acide ascorbique des cultivars 'Sabrina' (4.6 % et 55,5 mg 100 g^{-1} FW, respectivement) et 'Ventana' (4,0 % et 31,2 mg 100 g^{-1} FW, resp.). La fraise bio 'Ventana' avait un contenu supérieur en solides solubles et une activité anti-oxydante hydrophile plus forte, tandis que la tendance inverse était observée sur les teneurs en phénols totaux et en acide ellagique pour 'Ventana' et 'Sabrina', resp. **Conclusion.** La qualité des fraises et en particulier les composés bioactifs peuvent varier selon

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⋆ Corresponding author: youssef.rouphael@unina.it

le système de culture et le mode de gestion post-récolte. Les facteurs culturaux, y compris la gestion du système d'exploitation et le choix du cultivar, peuvent interagir de façon significative pour affecter les attributs de qualité des fraises.

Mots clés : Italie / fraise / *Fragaria × ananassa* / agriculture biologique / qualité du fruit / activité anti-oxydante / acide ascorbique / acide ellagique / composés phénoliques

1 Introduction

According to the Codex Alimentarius Commission, "organic agriculture is a holistic production system that promotes health and the sustainable development of agro-ecosystems by obeying biodiversity, biological cycles and soil biological activity using fertilizers of organic origin and renewable energy sources" [1]. The demand for organic products has increased rapidly in the last two decades, partially due to the general perception among consumers that organic foods are healthier and more nutritive than conventionally produced products [2]; although the scientific evidence is still insufficient to confirm or reject this assumption [3].

Strawberry is a major crop in Italy with about 3,700 ha under both open field (~700 ha) and protected cultivation (~3,000 ha). The major production areas of strawberry are located in Campania (833 ha), Veneto (633 ha) and Basilicata (509 ha). The Italian strawberry production is almost entirely obtained from conventional farming; however, in the last decade the area devoted to organic strawberry production has doubled from 15 to 34 ha [4]. Strawberry is considered an important fruit in the Mediterranean diet due to its high content of bioactive compounds, in particular antioxidants, which may have relevant biological activity [5]. For instance, strawberries are considered as a relatively potent anti-proliferative agent against the growth of human liver cancer cells HepG2 [6]. Moreover, strawberries are also appreciated by consumers because of their relatively high content of ellagic acid, an antioxidant that has been proposed to exert anticarcinogenic and antimutagenic effects [7, 8]. Strawberries also contain other antioxidants with health-promoting properties, such as vitamin C, anthocyanins, and other flavonoids such as quercetin and kaempferol [9, 10]. Concerning the quality parameters of organic strawberry, Olsson *et al.* [11] demonstrated that strawberry grown organically had a higher ratio of ascorbate to dehydroascorbate and higher total phenolics, ellagic acid and flavonol contents, indicating that organically grown strawberries might have a higher content of secondary metabolites than conventional strawberry. However, other studies [9, 12] reported that organic cultivation had no consistent effects on sugars, antioxidants, and macro- and micronutrients contents in strawberry compared with conventional farming. Therefore, it is premature to conclude that either organic or conventional fruits are healthier. Thus, additional research comparing these two farming management systems is needed to provide further information on this topic [13].

Strawberry is generally consumed as a fresh product or in processed forms, in particular as frozen fruits [14]. The large scale of frozen strawberry production could be associated with the short shelf life of the berries and the very broad scale of the use of frozen fruits in the industrial manufacturing of strawberry jam and juices [14, 15]. However, during the frozen food chain (freezing, frozen storage and subsequent thawing) strawberries could be subjected to quality changes such as the reduction of the antioxidant compound content [16]. Similarly, Garrote and Bertone [17] demonstrated that strawberry phenolics (*i.e.* ellagic acid, p-coumaric acid, quercetin and keampferol) are unstable and undergo deterioration during freezing and thawing processes.

Based on the above considerations, the aim of this study was to investigate the effects of the farming system (organic versus conventional) and postharvest treatment (fresh after harvest or thawed after storage for 6 months at −40 °C) on the physical, chemical and phytochemical components of two strawberry cultivars.

2 Materials and methods

2.1 Plant material and sampling

Two independent experiments were performed to assess the fruit quality of strawberry (*Fragaria × ananassa* Duch.) grown under conventional and organic management systems in Vitulazio-Caserta (41°09′46″ N latitude, 14°12′48″ E longitude) and in Sezze-Latina (41°30′33″ N latitude, 13°04′37″ E longitude). The 'Sabrina' and 'Ventana' strawberry cultivars were selected as the most representative cultivar used in the province of Caserta (Vitulazio) and Latina (Sezze), respectively. In both experiments, plants were grown in a cold greenhouse on clay soil with drip irrigation. The organic farming was conducted following the EU regulations (EC 834/2007) [18], whereas the conventional farming system was done according to the standard farming management practices.

Treatments were defined by a factorial combination of the two farming systems (conventional or organic), and the two postharvest storage treatments (fresh after harvest or thawed after frozen storage). In both experiments, treatments were arranged in a randomized complete block design with three replicates.

Chemical analyses were performed on a representative sample of strawberry fruits of both cultivars collected from each plot. Fruits were picked at commercial maturity (> 80% of the fruit surface showing red color) early in the morning and were immediately transferred to the laboratory for further analyses. Fruits were selected based on uniformity of size and the absence of physical damage and fungal infection.

Thirty fruits were sampled from each replicate and the total number of strawberry fruits analyzed in each experiment was 360. The fruits were water-washed and each sample was subdivided into two sub-samples of 15 fruits. One sub-sample

was used for analysis of firmness, surface color, titratable acidity, antioxidant activity, soluble solids, total sugars and bioactive compound contents. The other sub-sample was stored at −40 °C in a freezer for 6 months for analysis. Thawing was carried out at 8 °C for 10 h before analysis.

2.2 Color measurements

In both experiments, external color was measured on opposite sides of 15 fruits per experimental unit using a Minolta CR-300 Chroma Meter (Minolta Camera Co. Ltd., Osaka, Japan). The measuring aperture diameter was 8 mm, and the instrument was calibrated with a Minolta standard white reflector plate before sampling the berries. Samples were placed on a white background and single readings were taken with the hand-held unit on the upper surface of each strawberry midway between the apical and basal ends. The CIELAB color space parameters recorded were lightness (L*), chroma coordinates a* and b*, chroma (C*), and hue angle (h°) [19].

2.3 Physicochemical analysis

In both experiments, fruit firmness (kg cm^{-2}) was determined by penetration using an Ametek firmness tester (Ametek Hunter Spring Division, Hatfield, PA, USA) fitted with a 6-mm o.d. v-notched tip. On the extract obtained by liquefying and filtering the strawberry fruits, the total soluble solids (TSS) content of the juice was determined using an Atago N1 refractometer (Atago Co. Ltd., Tokyo, Japan) and expressed as °Brix at 20 °C. Acidity was determined by potentiometric titration with 0.1 M NaOH up to pH 8.1 using 10 mL juice and the results were expressed as percentage of citric acid in the juice. Reducing sugars were determined by reaction of a water-soluble portion of the sample with an excess of standard copper sulfate in alkaline tartrate (Fehling's) solution under controlled conditions of time, temperature, reagent concentration and composition, so that the amount of copper reduced is proportional to the amount of reducing sugars in the sample analyzed. Fruit juice pH was also determined. Fruits were dried in a forced-air oven at 60 °C for 72 h and weighed to determine the fruit dry matter (DM).

2.4 Hydrophilic and lipophilic antioxidant activities

Two different radical cation assays were used to determine the antioxidant activity of the hydrophilic (*i.e.* water-soluble) and lipophilic (*i.e.* lipo-soluble) fractions, on 0.2 g of lyophilized samples, extracted by distilled water and methanol, respectively. The hydrophilic antioxidant activity (HAA) was assessed using the N,N-dimethyl-p-phenylenediamine (DMPD) method [20]. The principle of the assay is that in the presence of a suitable oxidant solution DMPD forms a stable and colored radical cation (DMPD$^{.+}$). Antioxidant compounds which are able to transfer a hydrogen atom to DMPD$^{.+}$ quench the color and produce a discoloration of the solution which is proportional to their amount. The lipophilic antioxidant activity (LAA) was measured using the 2,2'-azinobis

3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method as described by Pellegrini *et al*. [21]. The principle of the assay is that the inhibitory response of the radical cation is proportional to the antioxidant concentration and the reaction is complete at the time point selected of 2.5 min. The HAA and LAA were determined by UV–Vis spectrophotometry. The absorbance of the solution was measured at 505 and 734 nm, respectively. HAA and LAA were expressed as mmol ascorbic acid (AA) and mmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per 100 g fresh weight, respectively [20,22].

2.5 Total ascorbic acid and phenolic contents

The total ascorbic acid, defined as ascorbic acid (AsA) and dehydroascorbate (DHA) acid, was assessed by spectrophotometric detection on fruit samples from the two experiments. The assay is based on the reduction of Fe^{3+} to Fe^{2+} by AsA and the spectrophotometric detection of Fe^{2+} complexes with 2,2'-dipyridyl [23]. DHA is reduced to AsA by pre-incubation of the sample with dithiothreitol (DTT). The absorbance of the solution was measured at 525 nm, and data were expressed as mg ascorbic acid on 100 g fresh weight.

The total phenolic content was determined in methanol extracts (1 g of freeze-dried strawberries were extracted in 20 mL of 60% methanol) according to the Folin-Ciocalteu procedure [24] using gallic acid as a standard. A 100-μL aliquot of the supernatant was combined with 500 μL of Folin-Ciocalteu reagent (Sigma Aldrich Inc., St Louis, MO, USA) and 400 μL of 7.5% sodium carbonate/water (w/v). The tubes were mixed for 15 s and then allowed to stand for 30 min at 20 °C. Absorption was measured at 765 nm using a UV-Vis spectrophotometer, and the result was expressed as mg gallic acid 100 g^{-1} dry weight.

2.6 Ellagic acid content

To determine the total amount of ellagic acid, the fruit samples (5 g) were extracted and hydrolyzed in 50% (v/v) aqueous methanol according to the procedure described by Hakkinen *et al*. [25]. The samples were heated to 85 °C for 20 h in 0.6 M HCl and 0.15% ascorbic acid. Samples were then centrifuged at 10,000 *g* for 5 min. The supernatant of each sample was diluted with distilled water to twice the volume, placed on a SepPak C18 column and eluted with 1.4 mL of methanol before analysis on a HPLC apparatus (Shimadzu LC 10, Shimadzu, Kyoto, Japan) equipped with a UV/Vis detector (mod. SPD-M10A 230 V) and a Prodigy ODS3 100 Å column (250 × 4.6 mm, particle size 5 μm) (Phenomenex, Torrance, CA, USA). The mobile phase was a linear binary gradient with solvent A (50 mM acetic acid and 5% (v/v) acetonitrile) and solvent B [acetonitrile/5% (v/v) MeOH] at a flow rate of 1 mL min^{-1}. Each chromatogram was recorded at 256 nm.

2.7 Statistical analysis

All data were statistically analyzed by ANOVA using the SPSS 20 software package (www.ibm.com/software/analytics/spss). To separate treatment means within each measured parameter, Duncan's multiple range test was performed

Table I. Effects of farming system and frozen storage on hunter color parameters L* (brightness), a* (+a* = red; -a* = green) and b* (+b* = yellow; -b = blue), hue (h°), Chroma (C*) of two strawberry cultivars 'Sabrina' and 'Ventana'.

Farming system	Storage	L*	a*	b*	h°	C*
cv 'Sabrina'						
Organic	Fresh	19.3	42.2 a	33.1	38	53.7 a
	Frozen	18.8	44.3 a	32.4	36.1	54.9 a
	Mean	*19.1*	*43.3*	*32.8*	*37.1*	*54.3*
Conventional	Fresh	17.6	44.5 a	30.1	34.1	53.8 a
	Frozen	14.1	38.1 b	24.2	32.4	45.2 b
	Mean	*15.9*	*41.3*	*27.2*	*33.3*	*49.5*
Significance						
Farming (F)		**	NS	**	**	**
Storage (S)		NS	NS	NS	NS	NS
F × S		NS	*	NS	NS	**
cv 'Ventana'						
Organic	Fresh	27.6	37.7	25.2	33.8	45.4
	Frozen	24.0	40.6	26.0	32.4	48.3
	Mean	*25.8*	*39.1*	*25.6*	*33.1*	*46.8*
Conventional	Fresh	15.0	28.1	16.3	30.2	32.5
	Frozen	14.0	33.9	16.1	25.4	37.5
	Mean	*14.5*	*31.0*	*16.2*	*27.8*	*35.0*
Significance						
Farming (F)		**	**	**	*	**
Storage (S)		*	*	NS	NS	NS
F × S		NS	NS	NS	NS	NS

Means for the two farming systems over the two postharvest storage treatments are reported in italic font. Within columns, means with different letters are significantly different according to Duncan's test ($P \leqslant 0.05$). NS, *, ** are non-significant or significant at $P \leqslant 0.05$ or 0.01, respectively.

at $P < 0.05$. Principal component analysis (PCA) using a correlation matrix was performed on quality attributes to assess relationships among variables and treatments, and also to determine which traits were the most effective in discriminating between the farming system and postharvest storage. PCA outputs include treatment component scores and variable loading to each selected component. The first two components PC1 and PC2 were selected for the ordination analysis, and the correlation between the original traits and the respective PC was calculated. Loadings >|0.6| indicate significant correlations between the original variables and the extracted components [26].

3 Results and discussion

3.1 Color

Strawberry surface color has been generally regarded by consumers as indicative of the overall fruit quality, and the intensity of the red hue in particular has been associated arbitrarily with sweetness [27, 28]. In both cultivars, the changes in Hunter color parameters were mainly due to the farming system and, to a lesser degree, to the effect of postharvest frozen storage. For the 'Sabrina' cultivar, no significant differences were observed between the fresh and thawed after

frozen storage fruits, whereas for the 'Ventana' cultivar, the brightness (L*) of fresh strawberry fruit (avg. 21.3) was 12.2% higher than the frozen ones (avg. 19.0), while an opposite trend was observed for the redness (a*) parameter, with the highest values recorded in frozen (average 37.2) compared with fresh berries (average 32.9) *(table I)*.

When averaged over the storage treatment, organic farming significantly increased the L*, a*, b*, h° and C* in 'Sabrina' by 20.1%, 4.8%, 20.5%, 11.4% and 9.7%, respectively, in comparison with those recorded in the conventional farming system *(table I)*. The Hunter color parameters were more pronounced in 'Ventana' since the L*, a*, b*, h° and C* values of strawberry fruit from organic farming were significantly higher by 77.9%, 26.1%, 58.0%, 19.0% and 33.7%, respectively, when compared with those observed in the conventional strawberry crop *(table I)*.

Overall, in both cultivars the color of organic fruit was brighter and more vivid (higher L* and C* values). The results reported in the current experiment are in contrast with those recorded by Crecente-Campo *et al.* [27], who observed that the color of organic strawberry fruit (cv. 'Selva') was darker, less vivid and tended to be redder (lower L*, C*, h°, values) in comparison with the conventional farming system. Explanations for this disagreement could be attributed to genetic, environmental and agronomic differences between

Table II. Effects of farming system and frozen storage on fruit mean weight (MW), dry matter (DM), firmness, juice pH, total soluble solid (TSS) content, titratable acidity (TA), and reducing sugars (RS) of two strawberry cultivars 'Sabrina' and 'Ventana'.

Farming system	Storage	MW (g fruit^{-1})	DM (%)	Firmness (kg cm^{-2})	pH	TSS (°Brix)	TA (%)	RS (%)
cv 'Sabrina'								
Organic	Fresh	11.0	9.1	1.3 a	3.5	7.3 ab	0.85	4.2
	Frozen	10.4	8.7	0.3 c	3.5	6.4 b	0.84	5.2
	Mean	*10.7*	*8.9*	*0.8*	*3.5*	*6.8*	*0.84*	*4.7*
Conventional	Fresh	16.2	8.8	1.1 a	3.7	7.7 a	0.72	4.9
	Frozen	10.4	8.0	0.6 b	3.7	5.3 c	0.76	4.0
	Mean	*13.3*	*8.4*	*0.8*	*3.7*	*6.5*	*0.74*	*4.5*
Significance								
Farming (F)		**	NS	NS	**	*	NS	NS
Storage (S)		**	NS	**	NS	**	NS	NS
F × S		NS	NS	*	NS	**	NS	NS
cv 'Ventana'								
Organic	Fresh	11.9	7.5	1.3 a	3.6	6.3 a	0.63	3.5
	Frozen	9.1	8.7	0.2 b	3.6	6.4 a	0.91	4.2
	Mean	*10.5*	*8.1*	*0.7*	*3.6*	*6.3*	*0.77*	*3.9*
Conventional	Fresh	17.8	6.2	1.3 a	3.7	6.5 a	0.50	4.4
	Frozen	12.8	6.2	0.3 b	3.7	5.0 b	0.71	3.7
	Mean	*15.3*	*6.2*	*0.7*	*3.7*	*5.8*	*0.60*	*4.0*
Significance								
Farming (F)		**	**	**	*	*	*	NS
Storage (S)		**	NS	**	NS	**	**	NS
F × S		NS	NS	NS	NS	**	NS	NS

Means for the two farming systems over the two postharvest storage treatments are reported in italic font. Within columns, means with different letters are significantly different according to Duncan's test ($P \leqslant 0.05$). NS, *, ** are non-significant or significant at $P \leqslant 0.05$ or 0.01, respectively.

the two experiments. Moreover, the color of 'Ventana' fruit became darker and redder after frozen storage. This was in agreement with the findings of Koyuncu [29], who reported similar color changes in the three strawberry cultivars 'Camarosa', 'Cavendish' and 'Chandler', subjected to storage at 0 °C.

3.2 Mean weight, firmness and flavor compounds

The fruit mean weight in both strawberry cultivars was significantly affected by the storage and farming system. For instance, the fruit mean weight was negatively affected by frozen storage since weight decreased in frozen fruits (*table II*). Moreover, organic strawberries had a lower mean weight, as previously reported by Abu-Zahra and Tahboub [30] for the cultivar 'Camarosa', although this may not always be true according to Baruzzi *et al.* [31] and Conti *et al.* [32], who analyzed different Italian strawberry cultivars. It is well established that organic materials (fish meal or powder, feather, alfalfa, soybean, bone, blood and meat meal) are less concentrated nutrient sources than conventional fertilizers and are characterized by low mineralization rates [33]. Therefore, the lowest fruit yield and fruit mean weight under organic farming could be attributed to a reduced availability

of nutrients, in particular N, at the right moment for the crop nutrient demand [32].

'Ventana' berries from organic farming were significantly smaller (by 31.3%) than those from conventional farming, but had significantly greater dry matter percentage (30.6%) (*table II*), which is in line with the findings of [34]. The greater dry matter percentage may be due to a higher proportion of cell wall content in the small-sized 'Ventana' organic berries compared with the large-sized organic fruits [35,36] as a result of the lower nitrogen concentration in organic fruit [37].

The fruit firmness, an important quality trait in determining consumer acceptability [38], was affected by the farming system and the postharvest frozen storage (*table II*). Significant interaction between the farming system and the frozen storage was observed in 'Sabrina'. The highest firmness values were recorded in fresh fruits under both organic and conventional management, whereas the lowest values were observed in frozen organic fruits. Strawberry is a soft fruit that suffers a rapid loss of firmness during ripening, which contributes greatly to its short shelf life [39]. In a recent study, Klat *et al.* [40] showed that postharvest life of fruits depends on their firmness, which is also related to fertilized achenes and thus dependent on successful pollination. Higher levels of plant hormones such as auxin and gibberelic acid

enhance firmness and thus shelf life by limiting the expression of fruit-softening proteins in particular expansions [41]. The firmness of the fruits in both cultivars reduced sharply during storage, mainly due to loss of cell wall material, which is more pronounced in the cortical tissue than in the pith tissue [42]. Furthermore, the fruit softening of strawberry fruit is due to their particular structure, characterized by large cells and thin cell walls [42]. Our results concerning frozen storage are in line with those of Ali *et al.* [43], who demonstrated that strawberry (cv. 'Sweet Charlie') firmness decreased significantly during postharvest storage at 0 °C for 15 days.

The juice pH in both cultivars was significantly affected by the farming system, with the highest values recorded in conventional farming (on average 3.7 *vs.* 3.5 in organic farming). The values of the juice pH recorded in the current experiment were consistent with the findings of Koyuncu [29] in several strawberry cultivars (with pH values ranging between 3.4 and 4.2). Contrarily to juice pH, the highest titratable acidity (TA) in the cultivar 'Ventana' was recorded in the organic farming system, whereas no significant difference among treatments was observed in TA in 'Sabrina'. This negative correlation between pH and titratable acidity could be expected, considering that fruit juice pH is determined primarily by organic acids [44]. Moreover, the titratable acidity recorded in 'Ventana' fruits subjected to frozen storage was significantly higher than that recorded at harvest, whereas no significant difference among treatments was observed in 'Sabrina' (*table II*). The increase in titratable acidity (e.g. citric acid) during frozen storage could be attributed to the changes in the permeability of the cell membrane, resulting in water leaving the cells and thus enhancing the solute concentrations [45].

The total soluble solid (TSS) content of the juice, which comprises soluble carbohydrates (mainly glucose and fructose), has been regarded by convention as a measure of sweetness in most fruits including strawberry. In the current experiment, the TSS content in both cultivars was significantly affected by the farming system, frozen storage and their interaction, with the lowest values recorded in frozen fruits from the conventional system (*table II*). Both cultivars were found to be significantly different in their TSS contents, with the highest values recorded in 'Sabrina' (average 6.6 °Brix vs. 6.0 °Brix in 'Ventana'). When averaged over the farming system, the TSS content decreased sharply in both cultivars after frozen storage for 6 months. Except for 'Ventana' from organic farming, the TSS content decreased in both cultivars after frozen storage for 6 months. Because of the highly freezable water content in berries, freezing implies significant cellular damage, leading to significant losses in product quality (*i.e.* soluble solids) [46]. Finally, irrespective of storage, the organic management resulted in higher values of soluble solids in both cultivars (*table II*). These results are in agreement with several published studies [12, 30, 32, 47] which found that organic fruits have a higher TSS content than conventionally grown fruits. The higher TSS recorded in 'Ventana' grown organically could be due to lower fruit water content (*i.e.* greater dry matter percentage), as observed in previous studies [34, 36]. Furthermore, the higher availability of N in the conventional farming may also explain the lower TSS in berries, as in

Cantliffe *et al.* [43], and shows that increasing amounts of N will decrease strawberry fruit TSS.

3.3 Antioxidant activity and bioactive compounds

In the last two decades, antioxidant activity and bioactive compounds found in vegetables have generated significant interest among consumers and researchers due to their health-benefit properties [49]. In the current experiment, the effects of the farming system and postharvest frozen storage on the hydrophilic (HAA) and lipophilic (LAA) antioxidant activities were more pronounced in 'Ventana' than in 'Sabrina'. The HAA values of the frozen 'Sabrina' fruits (18.9 mmol ascorbic acid eq 100 g^{-1} FW) were significantly higher (29.4%) than those recorded in fruits analyzed fresh after harvest (14.6 mmol ascorbic acid eq 100 g^{-1} FW) (*table III*), whereas the lowest HAA values in 'Ventana' were observed in conventional farming without storage (*table III*). Moreover, no significant differences among treatments were recorded for LAA in 'Sabrina' (18.4 mmol Trolox eq 100 g^{-1} FW), whereas LAA in 'Ventana' was significantly higher in conventional in comparison with organic, and in fresh than in frozen fruits (*table III*).

Total ascorbic acid has long been considered an important nutritional compound of strawberries [50], since it offers potential benefits to human health for protection against diseases [51]. No significant differences among treatments were recorded in total ascorbic acid (AA) in 'Ventana', while 'Sabrina' was significantly affected by postharvest frozen storage, with the highest values recorded in fresh fruits compared with the fruits thawed after frozen storage (*table III*). Similar results were found by Perez *et al.* [52] and Koyuncu [29] in two studies related to the storage of different strawberry cultivars at 2 and 0 °C, respectively. Similarly, Lisiewska and Kmiecik [53] observed that freezing resulted in a small decrease in the vitamin C content, which was reduced by 15−18% in broccoli and by 6−13% in cauliflower after 12 months of frozen storage at −30 °C. Our results also demonstrated that the cultivation system did not significantly affect the total ascorbic acid content in both cultivars. The literature on this topic is inconclusive. For instance, Asami *et al.* [54] studied the 'Northwest Totem' strawberry variety and demonstrated that the AA contents in organically grown samples were significantly higher (32.6 mg 100 g^{-1} FW) than the contents for conventionally grown fruits (27.1 mg 100 g^{-1} FW). Similarly, Jin *et al.* [13] indicated that the AA content was higher in organically cultivated (avg. 1.9−2.7 mmol kg^{-1} FW) versus conventionally cultivated (1.3−2.3 mmol kg^{-1} FW) 'Earliglow' and 'Allstar' strawberries. However, Hakala *et al.* [55] indicated that organic cultivation had no effect on the vitamin C content of the 'Honeoye' (62.5 mg 100 g^{-1} FW), 'Jonsok' (45.0 mg 100 g^{-1} FW) and 'Polka'(52.5 mg 100 g^{-1} FW) varieties, whereas Cardoso *et al.* [56] showed that the AA content was significantly lower in organic strawberries (30.7 mg 100 g^{-1} FW) than in conventional strawberries (42.4 mg 100 g^{-1} FW).

No significant differences among treatments were recorded for total phenol content in 'Sabrina' (106.4 mg gallic acid 100 g^{-1} DW), whereas a significant effect of the farming

Table III. Effect of farming system and frozen storage on hydrophilic (HAA) and lipophilic (LAA) antioxidant activities, total ascorbic acid (AA), total phenols (TP) and ellagic acid contents of two strawberry cultivars 'Sabrina' and 'Ventana'.

Farming system	Storage	HAA mmol ascorbic acid eq 100 g⁻¹ FW	LAA Mmol Troloxeq. 100 g⁻¹ FW	AA mg 100 g⁻¹ FW	TP mg gallic acid 100 g⁻¹ DW	Ellagic acid mg 100 g⁻¹ DW
cv 'Sabrina'						
Organic	Fresh	15.7	19.7	72.8	106.8	55.0
	Frozen	19.6	17.7	41.8	104.2	49.8
	Mean	*17.7*	*18.7*	*57.3*	*105.5*	*52.4*
Conventional	Fresh	13.6	18.1	58.0	100.5	60.4
	Frozen	18.2	18.1	49.3	114.2	51.9
	Mean	*15.9*	*18.1*	*53.7*	*107.3*	*56.2*
Significance						
Farming (F)		NS	NS	NS	NS	*
Storage (S)		**	NS	**	NS	**
F × S		NS	NS	NS	NS	NS
cv 'Ventana'						
Organic	Fresh	24.9 a	15.2	23.7	96.7	55.6
	Frozen	19.7 b	12.6	21.8	88.4	44.9
	Mean	*22.3*	*13.9*	*21.8*	*92.6*	*50.3*
Conventional	Fresh	12.0 c	21.7	38.9	111.6	57.0
	Frozen	24.7 a	17.6	40.5	98.5	46.7
	Mean	*18.4*	*19.7*	*39.7*	*105.0*	*51.9*
Significance						
Farming (F)		**	**	NS	*	NS
Storage (S)		**	*	NS	NS	**
F × S		**	NS	NS	NS	NS

Means for the two farming systems over the two postharvest storage treatments are reported in italic font. Within columns, means with different letters are significantly different according to Duncan's test ($P \leqslant 0.05$). NS, *, ** are non-significant or significant at $P \leqslant 0.05$ or 0.01, respectively.

system was observed in 'Ventana', since an increase of 13.4% in total phenolic content was observed on fruits harvested from the conventional system (*table III*). Similarly to the total phenols, the ellagic acid, a naturally occurring polyphenolic secondary metabolite that accumulates in strawberry fruit [57], was affected by the cultivation management system but only in 'Sabrina'. The ellagic acid content in 'Sabrina' recorded in the conventional farming was significantly higher by 7.2% than in organic strawberries (*table III*). Finally, our results showed that the ellagic acid content significantly decreased by 11.9% and 18.6% in 'Sabrina' and 'Ventana', respectively, when fruits were stored at −40 °C for 6 months. This is in agreement with the findings of Hakkinen and Torronen [9], who reported a 40% decrease in ellagic acid contents in strawberry during 9 months of storage in a freezer, and also in line with the results of de Ancos *et al.* [58], who showed a degradation of ellagic acid (19−21%) in raspberry after 365 days at −20 °C. It is generally proved that ellagic acid is a phenolic compound strongly linked to the cell wall [59]. The losses of ellagic acid in both berries during storage at −40 °C for 6 months could be related to a severe cellular disruption which could be produced by release of oxidoreductase ionic forms of the enzyme polyphenoloxidase (PPO) linked to the cell wall [58].

3.4 Principal component analysis

As defined by Lawless and Heymann [60], "PCA is a multivariate technique used to summarize and extract trends when many variables are used". PCA formulates new variables called principal components (PC), which summarize the variability of the original variables better and are correlated with them. The first three PCs were associated with Eigen values > 1, and explained 84.5% of the total variance, with PC1 accounting for 44.3% and PC2 for 30.0% (*table IV*). PC1 was positively and significantly correlated with C*, h°, a*, b*, fruit dry matter, total soluble solids content and titratable acidity (*table IV*). PC2 was positively correlated with lipophilic antioxidant activity, total ascorbic acid, total phenols and ellagic acid content, and negatively correlated with hydrophilic antioxidant activity. Moreover PC3, which explained 10.1% of the variance, was negatively correlated with L* (*table IV*).

Generally, the first two PCs account for meaningful variance; therefore only PC1 and PC2 were retained and interpreted (*figures 1A, B*). The loading plot (*figure 1A*) illustrates the relationships among variables (*i.e.* quality attributes), where two vectors with an angle > 90° are positively correlated and two vectors with an angle > 90° are negatively

Table IV. Correlation coefficient for each quality attribute with respect to the first three principal components PC1, PC2, and PC3 eigenvalues, relative and cumulative proportion of total variance.

Trait	Principal components (PC)[1]		
	PC1	PC2	PC3
L^*	0.473	–0.556	**–0.615**
C^*	**0.981**	0.034	0.188
$h°$	**0.821**	0.310	–0.165
a^*	**0.968**	–0.109	0.203
b^*	**0.968**	0.143	0.071
Fruit dry matter	**0.963**	–0.049	–0.005
Total soluble solids	**0.652**	0.471	–0.429
Reducing sugars	0.587	0.279	0.064
Titratable acidity	**0.661**	–0.489	0.461
Hydrophilic antioxidant activity	–0.039	**–0.852**	0.224
Lipophylic antioxidant activity	–0.362	**0.882**	0.145
Ascorbic acid	0.324	**0.764**	0.406
Total phenols	–0.290	**0.781**	0.280
Ellagic acid	0.148	**0.725**	–0.471
Eigenvalue	6.21	4.20	1.42
Percentage of variance	44.3	30.0	10.1
Cumulative variance	44.3	74.3	84.5

[1]The bold values indicate the most relevant characters for each principal component.

correlated [44]. For instance, ellagic acid content was positively correlated with total ascorbic acid and, to a lesser degree, total soluble solids and h°, whereas total soluble solids content was highly correlated with reducing sugars. Similarly, total phenol content was more strongly correlated with lipophilic antioxidant activity than ellagic acid content. A negative correlation between total phenols and titratable acidity was also observed (*figure 1A*).

The score plot of components 1 and 2 (*figure 1B*) separates treatments into four groups. The positive side of PC1 (quadrants 2 and 4) included fruits of 'Sabrina' and 'Ventana' at harvest and thawed after frozen storage from organic farming management, but also fresh conventional 'Sabrina' fruits. The treatments of the upper right quadrant, in particular organic and conventional 'Sabrina' fruits at harvest, were characterized by high total soluble solids, ascorbic acid content and h°, whereas the cluster in the lower right quadrant represents fruits characterized by higher brightness (L^*) (*figure 1B*).The negative side of PC1 (quadrants 1 and 3) corresponded to conventional fruits of 'Ventana' at harvest and thawed after frozen storage, but also to conventional 'Sabrina' stored at −40 °C for 6 months. The treatments of the upper left quadrant (1) were characterized by high lipophilic antioxidant activity and total phenol content, whereas those of the lower left quadrant (3) had relatively the lowest fruit quality among the treatments (*figure 1B*). The PCA carried out in the current experiment demonstrated the ability of this analysis to track the effects of farming management and postharvest storage fruit quality of two strawberry cultivars and could constitute the basis of future strategies to optimize quality [61].
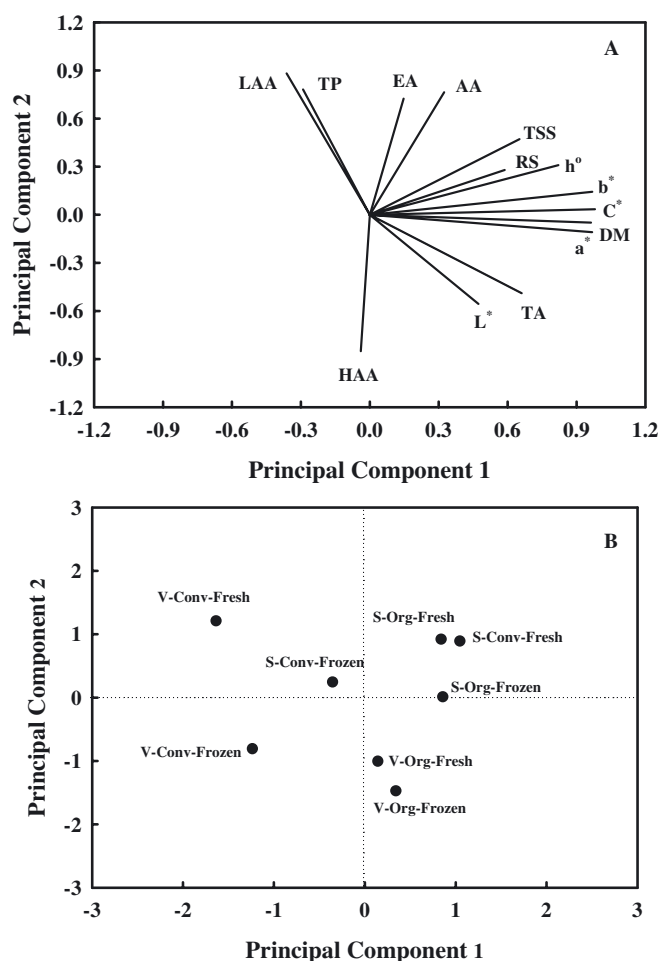
Figure 1. (A) Principal component loading plot and (B) scores of principal component analysis of physicochemical fruit traits of two strawberry cultivars (S, 'Sabrina'; V, 'Ventana') as a function of farming system management (Org, organic; Conv, conventional), and postharvest storage (Fresh, at harvest; Thawed after frozen storage at −40 °C for 6 months). L*, lightness; C*, chroma; h°, hue angle; a*, redness; b*, yellowness; DM, fruit dry matter; TSS, total soluble solid content; RS, reducing sugars; TA, titratable acidity; HAA, hydrophilic antioxidant activity; LAA, lipophilic antioxidant activity; AA, ascorbic acid; TP, total phenols; EA, ellagic acid.

4 Conclusion

As a summary, the results of the two trials showed that the skin lightness, fruit firmness, total soluble solids and ellagic acid contents scored higher at harvest than thawed after storage at −40 °C for 6 months. Postharvest frozen storage positively affected the hydrophilic antioxidant activity, leaving reducing sugars and total phenol contents without significant alterations. Our results also demonstrated that for both cultivars, the color of organic fruits was brighter and more vivid (higher L* and C* values). Strawberries had higher HAA in organic 'Ventana' and higher ellagic acid content in conventional 'Sabrina', indicating that cultivar characteristics should be taken into consideration in choosing a cultivar for a specific farming system. This could be critical to fully exploit the potential of organic horticulture.

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