



# Article Stability of Strawberry Fruit (*Fragaria x ananassa* Duch.) Nutritional Quality at Different Storage Conditions

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Abstract: Strawberry fruit is a very rich source of vitamins and phenolic compounds, which determine its nutritional properties. Strawberries are a highly perishable non-climacteric fruit, and their perishable nature can lead to physical and chemical damage during storage. Therefore, the large market of fresh fruit relies on the capacity of fast distribution and marketing under a continuous cold-storage chain. In this study, we applied different cold-storage temperatures (domestic -20 °C and industrial -80 °C) on different treatments (whole fruits and dried fruits) of three strawberry cultivars (Arianna, Francesca, and Silvia), for up to seven months, and evaluated the influence of different storage conditions and lengths on the stability of the fruits' nutritional compounds (vitamin C, phenolic acids, anthocyanins, and folate). The results show that the nutritional quality of the fruits was significantly affected by storage temperature (with -80 °C storage preserving more nutritional compounds), while storage time did not greatly affect the composition of the nutritional compounds in the whole or dried fruits. Oven drying the fruits dramatically affected their vitamin C content, almost completely degrading this compound (from 731.8 to 23.2 mg/kg at time 0 for fresh Arianna fruit, the cultivar with the highest amount). The amount of folate was increased during storage (from 126.17 at time 0 to 190.61  $\mu$ g/kg at time 7 for fresh whole Arianna fruit). The interesting results obtained in this study are worth considering in future studies, to better plan fruit-storage conditions and time, for maintaining better fruit nutritional quality.

Keywords: folate; polyphenols; storage; strawberry; vitamin C

## 1. Introduction

Strawberries are the most cultivated berry fruit worldwide, with an annual production of 13.3 million tons, on a surface area of 522,527 ha [1]. Consumer health expectations are linked to the intrinsic characteristics of the product, such as the presence of bioactive compounds with a nutraceutical effect. Many epidemiologic studies have shown that a diet rich in fruits and vegetables is often associated with a lower incidence of several chronic pathologies, including obesity, infections, cancer, and cardiovascular and neurologic diseases [2,3]. Berries, including strawberries, have an important role among fruits because of their high phytochemical content [4,5]. Numerous scientific studies confirm that the strawberry contains bioactive molecules with antioxidant power, such as ascorbic acid, polyphenolic compounds such as ellagic acid, ferulic acid, and some flavonoids (anthocyanins, catechins, phenolic acids, etc.). These compounds exhibit a nutraceutical effect, exerting beneficial and protective properties on the human body [6].

Strawberries are a highly perishable non-climacteric fruit, which can lead to physical damage during storage and transportation. Therefore, the large market of fresh fruit relies on the capacity of fast distribution and marketing under a continuous cold-storage chain. Fruit overproduction, especially during off-season periods, is addressed mostly toward the processing industry to produce bakery products such as jam, jellies, juice, puree, flavor additives, etc. [4,7], with a consequence being a price reduction for the product.



Citation: Qaderi, R.; Mezzetti, B.; Capocasa, F.; Mazzoni, L. Stability of Strawberry Fruit (*Fragaria x ananassa* Duch.) Nutritional Quality at Different Storage Conditions. *Appl. Sci.* 2023, *13*, 313. https://doi.org/ 10.3390/app13010313

Academic Editor: Maria Kanellaki

Received: 1 December 2022 Revised: 20 December 2022 Accepted: 25 December 2022 Published: 27 December 2022



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Strawberries' post-harvest decay can be due to physical, physiological, or pathological factors that may happen in the pre-harvest period (and then take place during storage) or directly in the post-harvest period, and their shelf life is diminished by firmness loss, fruit desiccation, and the growth of spoilage microorganisms [7].

Freezing is a simple but common and effective method for long-term preservation and storage of fruits and vegetables, while maintaining many of their fresh-like qualities [8,9]. In addition, freezing is less destructive than other available preserving methods [10]. Given the nutritional value of fruits and vegetables, it is essential that bioactive compounds and nutrients are considered healthy when frozen and stored. These methods support the food supply chain and producer income when fresh food is not available. Long-term storage is becoming a more common in-home consumer behavior, and labs freeze food prior to analysis, both for convenience. Analytical labs and home kitchens typically have -20 °C freezers. Although -80 °C can be used in a laboratory, this equipment is expensive and has high operating costs, mainly due to energy consumption [11]. The freezing rate is the most important factor in the freezing process to prevent fruit-tissue damage, loss of bioactive compounds, and drip loss in thawing. Faster freezing results in small ice crystals and better frozen-fruit quality [12]. The effect of freezing fruits on microbial and enzymatic activity can influence the chemical composition, but storage temperature and storage period could also have an effect on the chemical composition of the strawberry [13].

Dehydration is another method for long-term preserving and increases the shelf life of delicate fruits. Oven drying fruits has been the most common preserving method used for many years [14], and, in the past few decades, considerable efforts have been made to understand the chemical and biochemical changes that occur during dehydration and to develop methods for preventing undesirable quality losses. Drying reduces water activity, and avoids microbial growth and deteriorative chemical reactions. The effects of heat on microorganisms and on the activity of enzymes are also important when drying fruits [15].

In this study, we evaluate the effect of fruit treatment on whole fruits and dried fruits (WF and DF) at different storage times (from 0 to 7 months) and temperatures (-20 and -80 °C) on the nutritional quality (vitamin C, anthocyanins, phenolic acids, and folate) of three strawberry cultivars.

#### 2. Materials and Methods

#### 2.1. Plant Material

Three commercial cultivars (Arianna, Francesca, and Silvia) were planted in July 2020 in non-fumigated soil in "P. Rosati" experimental farm of Università Politecnica delle Marche, sited in Agugliano (Ancona, Italy), with the following main characteristics: pH 7.9, active calcium 9%, and texture composed of 40% clay, 25% sand, and 35% silt, following the procedure described in Mezzetti et al. [16]. Plants were grown in open field conditions according to the plastic hill culture production system. Fruit samples were harvested at fully red stage, at the second, third, and fourth main seasonal pickings [7], and immediately treated according to the experimental storage design. The environmental data of the picking season (April–June 2021) were registered and are reported in Table 1.

**Table 1.** Monthly rainfall (sum) and average daily maximum, average, and minimum temperatures registered in April, May, and June 2021.

Month	Rainfall (mm)	<b>Maximum Temperature (°C)</b>	Average Temperature (°C)	Minimum Temperature (°C)
April	33.0	17.9	12.2	7.1
Мау	23.8	24.1	18.2	12.7
June	7.8	30.8	25.0	19.1

For all cultivars analysed, two fruit treatments were taken into consideration: whole fruits (WF) and oven-dried fruits (DF). Regarding the fruit treatments, for the WF, we collected fresh ripe fruit and directly put them into a normal -20 °C domestic refrigerator (Zoppas, Vittorio Veneto, Italy) and a laboratory -80 °C refrigerator (HFU B series, Thermo Fisher Scientific, Milan, Italy). For the DF, after the collection of fruit from the field, we put them in a laboratory oven (Pbi international, Milan, Italy) at 65 °C for 1 week. The loss of water and the complete dryness of fruits was monitored by checking the fruit weight at intervals of 24 h, until no further weight reductions were detected. Then, dried fruits were placed at -20 °C and -80 °C, as indicated before for WF.

Fruits were stored for five different storage times: 0, 1, 3, 5, and 7 months after the harvest day. During each storage time (except at 0, when the fruits were immediately analyzed), fruits of three cultivars were stored at -20 °C and -80 °C temperature conditions. For each combination of cultivar/storage time/treatment/storage temperature, three repetitions of 5 fruits were made. A summary of the experimental storage design is described in Table 2.

**Table 2.** Storage experimental design applied to each of the three studied cultivars. WF: whole fruit; DF: dried fruit.

Storage Time	Fruit Treatment	Storage Temperature	Number of Fruits
0.M	WF	_	5
0 101	DF		5
	1A/E	-20	5
1 M	۷۷۲	-80	5
	DE	-20	5
	DF	-80	5
	WF	-20	5
3 M		-80	5
	DF	-20	5
		-80	5
	W/E	-20	5
5 M	VV1	-80	5
	DF	-20	5
		-80	5
	W/F	-20	5
7 M	VVI	-80	5
	DE	-20	5
	DF	-80	5

For the analyses of fruit nutritional quality, the WF and DF were extracted and analyzed after 1, 3, 5, and 7 months of refrigerated storage; for the "time 0", WF were immediately extracted and analyzed after collection, while DF were immediately extracted and analyzed after oven-drying. The analyzed nutritional parameters were anthocyanins, phenolic acids, vitamin C, and folate. All the parameters were analyzed by HPLC-UV-FD.

#### 2.3. Methanolic Extraction

Fruit extracts were prepared as described by Diamanti et al. [17]. First, 10 g of WF and 1 g of DF were homogenized with Ultraturrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik, Staufen, Denmark) in 20 mL of methanol and agitated for 30 min in the dark. The suspension was centrifuged at 4500 rpm for 10 min at 4 °C, the supernatant was collected, and the pellet of the fruits was extracted for a second time by adding another 20 mL of methanol and repeating the procedure. The second supernatant was added to the first one and then immediately injected to HPLC.

Vitamin C of fruits was extracted with an ultrasound-assisted extraction protocol, as described by Tulipani et al. [18]. The extraction was carried out with the use of an ultrasound bath (Bioblock/ELMA 88155, Stuttgart, Germany), an instrument that generates ultrasound waves inside a tank containing water, using high frequency electric current produced by a generator. The process is useful to speed up the dissolution of solutes in certain solvents.

The analysis requires homogenizing 1 g of frozen strawberry for WF fruit and 0.1 g for DF with an aliquot of 4 mL taken from the extraction buffer solution, containing 5% metaphosphoric acid and 1 mM DTPA, followed by 5 min of sonication and centrifugation at 4000 rpm for 10 min at 4 °C. The supernatants obtained from each sample were filtered (filter pore size 0.45  $\mu$ m) and inserted into a vial to perform analysis on an HPLC system.

#### 2.5. Extraction of Vitamin B9 (Folate)

Following the method described by Mezzetti et al. [19], with slight modifications, 8 mL of the extracting solution (0.1 mol/L sodium acetate containing 10% (w/v) sodium chloride, 1% (*w*/*v*) ascorbic acid, and 0.1% 2-mercaptoethanol) were pipetted in 2 g of the frozen strawberry for WF samples and 0.2 g for DF samples, then homogenized with Ultraturrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik, Staufen, Denmark). The falcon tube was loosely capped, boiled in water for 12 min, and rapidly cooled in the freezer for 10 min. Hog kidney folate conjugate enzyme was prepared, and about 1.5 mL of the enzyme was added to the cooled solution and incubated in a shaking oven at 37 °C for 3 h. Afterwards, the enzyme was inactivated by boiling in water for 5 min followed by cooling for 10 min in the freezer. The samples were then centrifuged at 4500 rpm for 30 min at 4  $^{\circ}$ C, and the supernatant was transferred into a new labeled falcon tube. Moreover, another 8 mL of the extracting solution was added to the resulting pellet and centrifuged again for 30 min. The second supernatant was added to the first one, then extracting solution was added to top up the supernatants to 25 mL. The final supernatant of 25 mL was then filtered using 0.45 µm filter pore size, 25 mm inner diameter, nylon disposable syringe filters, and the filtrates were purified through solid-phase extraction on strong anion-exchange isolate cartridges, as described by Iniesta et al. [20].

## 2.6. HPLC Determination of Vitamin C Content

Vitamin C content was measured as described in Helsper et al. [21]. Extracts were subjected to HPLC analysis after the extraction procedure. The HPLC system comprised a Jasco PU-2089 plus controller (Jasco, Easton, MD, USA), a Jasco UV-2070 plus ultraviolet (UV) detector (Jasco Easton, MD, USA) set at an absorbance of 260 nm, and an autosampler Jasco AS-4050 (Jasco, Easton, MD, USA). The HPLC column used was Ascentis Express C18  $150 \times 4.6$  mm (Supelco, Bellefonte, PA, USA), protected by a Phenomenex  $4.0 \times 3.0$  mm C18 ODS guard column (Phenomenex, Torrance, CA, USA). The gradient program consisted of two mobile phases: A (50mM phosphate buffer with pH 3.2) and B (Acetonitrile), which started with 100% of A until 6 min, then decreased to 50% for 2 min, and again increased to 100% until the end.

The quantification of vitamin C content was carried out through calibration curve prepared by running standard concentration of vitamin C, and the results were expressed as mg vit-C per 1 kg fresh weight of strawberries (mg/kg FW).

## 2.7. HPLC Determination of Phenolic Acid Content

Phenolic acids were analyzed as previously described in Schieber et al. [22] and Fredericks et al. [23]. The HPLC system comprised a Jasco PU-2089 plus controller (Jasco, Easton, MD, USA), a Jasco UV-2070 plus ultraviolet (UV) detector (Jasco Easton, MD, USA), and an autosampler Jasco AS-4050 (Jasco, Easton, MD, USA). The HPLC UV detector was set at 320 nm and the column used was an Aqua Luna C18 250  $\times$  4.6 mm (Phenomenex, Torrance, CA, USA) protected by a Phenomenex 4.0  $\times$  3.0 mm C18 ODS guard column

(Phenomenex, Torrance, CA, USA). The gradient program consisted of two mobile phases: A (2% Acetic acid) and B (acetic acid, acetonitrile and  $H_2O$  1:50:49). It started with 55% A and 45% B for 50 min, followed by 10 min 100% of B, and then decreased to 10 % B until the end.

For the quantification of phenolic acid content, external chlorogenic acid (CHA), caffeic acid (CA), and ellagic acid (EA) calibration curves were used. Values were expressed as mg corresponding to phenolic acid per kilogram of fresh weight of strawberries (mg/kg FW).

## 2.8. HPLC Determination of Anthocyanin Content

Anthocyanin content was analyzed following the method of Fredericks et al. [23]. The HPLC system comprised a Jasco PU-2089 plus controller (Jasco, Easton, MD, USA), a Jasco UV-2070 plus ultraviolet (UV) detector (Jasco Easton, MD, USA), and an autosampler Jasco AS-4050 (Jasco, Easton, MD, USA). The compounds were separated on an Aqua Luna C18 (2) ( $250 \times 4.6 \text{ mm}$ ) reverse-phase column with a particle size of 5 µm (Phenomenex, Torrance, CA, USA) protected by a Phenomenex 4.0 × 3.0 mm C18 ODS guard column (Phenomenex, Torrance, CA, USA), and monitoring was performed at 520 nm. The gradient program consisted of two mobile phases: A (formic acid, acetonitrile, and H<sub>2</sub>O 10:3:87) and B (formic acid, acetonitrile, and H<sub>2</sub>O 10:50:40). It started with 75% A for 10 min, decreased to 69% A for 5 min, decreased again to 60% A for 5 min, and later continued 50% A for 10 min, followed by 90% A for 16 min.

Anthocyanins were quantified using calibration curves made with external standards of cyanidin-3-glucoside, pelargonidin-3-glucoside, and pelargonidin-3-rutinoside, and were calculated as mg per 1 kg of fresh weight of strawberries (mg/kg FW).

## 2.9. Quantification of Folate Content

Folate was quantified using the HPLC as cited by Stralsjo et al. [24]. The HPLC system comprised a pump model Jasco PU- 2089 (Jasco, Easton, MD, USA), a fluorescence detector (FLD) Jasco FP-2020 Plus (Jasco, Easton, MD, USA) set at wavelengths of 290 nm excitation and 360 nm emission, and an autosampler Jasco AS-4050 (Jasco, Easton, MD, USA). The analytical column was a Luna C18,  $250 \times 4.6$ ,  $5 \mu$ m (Phenomenex, Torrance, CA, USA), protected by a Phenomenex 4.0  $\times$  3.0 mm C18 ODS guard column (Phenomenex, Torrance, CA, USA). Quantification of folate content was determined through a calibration curve prepared by running standard concentrations of 5-methyl-tetrahydrofolic acid (5-CH<sub>3</sub>-H<sub>4</sub> folate). The gradient program consisted of two mobile phases: A (30 mM phosphate buffer with 2.3 pH) and B (Acetonitrile). It started with 94%A for 8 min, then decreased to 75% A for 27 min, and increased again to 94% A for 15 min.

Results are expressed as  $\mu$ g 5-CH<sub>3</sub>-H<sub>4</sub> folate per 1 kg of fresh weight of strawberries ( $\mu$ g 5-CH<sub>3</sub>-H<sub>4</sub>folate/kg FW).

## 2.10. Data Analyses

The results are presented as the values  $\pm$  standard error and were subjected to oneway analysis of variance (ANOVA), at a confidence level of 95%. Significant differences were calculated according to Tukey's tests, and differences at p < 0.05 were considered to be significant. A correlation matrix has also been developed among the nutritional parameters to check their inter-relationship, with p < 0.05. Statistical analyses were performed by using Statistica 7 software (StatSoft, TIBCO Software, Palo Alto, CA, USA).

### 3. Results and Discussion

## 3.1. Vitamin C

The vitamin C amounts of the Arianna, Francesca, and Silvia fruits at different storage times with different treatments are shown in Table 3. The highest vitamin C amount was detected in Arianna WF (731.8 mg/1 kg FW) at time 0, and the oven-drying process caused a high loss of total vitamin C (almost 97% at time 0 for DF). The vitamin C amount in the Arianna and Silvia WF during storage at -20 °C decreased from 1 M until the end

of storage, with the lowest amount detected at 5 M (436.1 mg/kg FW and 388.1 mg/kg FW, respectively). However, in Francesca WF, vitamin C slightly increased after 1 M (599.8 mg/kg FW) and was maintained until the end of storage. Regarding the DF in -20 °C storage, there was a slight increasing trend in the vitamin C amount for all cultivars until the end of storage, but the values remained dramatically lower than those of the WF.

**Table 3.** Average of vitamin C content (mg/kg FW) in fruits of Arianna, Francesca, and Silvia cultivars. The data are expressed as mean  $\pm$  standard error. Different lowercase letters indicate significant differences for  $p \le 0.05$  in each cultivar during storage. Different uppercase letters indicate significant differences for  $p \le 0.05$  for all cultivars for a specific storage time. WF: whole fruit, DF: dried fruit. 0 M: 0 months of storage; 1 M: 1 month of storage; 3 M: 3 months of storage; 5 M: 5 months of storage, 7 M: 7 months of storage.

Storage Time	Storage Temperature	Treatment	Arianna	Francesca	Silvia
0 M		WF	$731.8\pm6.5aA$	$580.4\pm3.6 \mathrm{fB}$	$519.3 \pm 0.3$ cC
0 101		DF	$23.2\pm0.3$ jD	$18.8\pm0.3$ mD	$16.5 \pm 0.3$ lD
	-20	WF	$650.6\pm3.9\text{bB}$	$599.8\pm2.8 \text{dC}$	$402.2 \pm 1.8$ gE
1 \ /	-80	WF	$737.4 \pm 4aA$	$736 \pm 2aA$	$592.1\pm0.9a\mathrm{D}$
1 IVI	-20	DF	$26.8\pm0.7 \mathrm{jF}$	$22.8\pm0.4 \mathrm{lmF}$	$16.4 \pm 0.3$ lG
	-80	DF	$27.9 \pm 0.1 \mathrm{jF}$	$25.1\pm0.3 ijklF$	$14.6\pm0.1\text{lG}$
	-20	WF	$554.4\pm0.1 \text{dD}$	$594.3\pm3.9\mathrm{eB}$	$423.4\pm0.2\mathrm{fF}$
2 M	-80	WF	$571.7 \pm 13.4 \mathrm{cC}$	$609.5\pm1.6\mathrm{cA}$	$523.7 \pm 2.3$ cE
3 IVI	-20	DF	$24.2 \pm 0.3$ jHI	$28\pm0.1$ ijkH	$19.4 \pm 0.2$ lHI
	-80	DF	$93.5 \pm 5.3 hG$	$24.3 \pm 0.3$ jklHI	$14.1\pm0.1\mathrm{lI}$
	-20	WF	$436.1\pm0.8 \mathrm{gD}$	$517.2\pm0.2\mathrm{gA}$	$388.1 \pm 0.3 hF$
БМ	-80	WF	$507.1 \pm 0.5 \mathrm{eB}$	$500.3 \pm 0 hC$	$430.1\pm0.1\mathrm{eE}$
J IVI	-20	DF	$25.4\pm0.2$ jI	$23.1 \pm 0.2$ klmI	$39.3\pm0.8$ jG
	-80	DF	$29.2\pm0.7 \text{jH}$	$30\pm0.7iH$	$31.3\pm2.3 \text{kH}$
	-20	WF	$544.6 \pm 1.2 \text{dC}$	$576.8\pm1\mathrm{fB}$	$509.4\pm0.3$ dD
7 M	-80	WF	$473\pm0.5\mathrm{fE}$	$664.1 \pm 1.5 \text{bA}$	$537.5 \pm 8.2 \mathrm{bC}$
/ IVI	-20	DF	$46.3\pm7.6\mathrm{iF}$	$29.3\pm0.5$ ijG	$26.7\pm0.1 kG$
	-80	DF	$27.3 \pm 1 \mathrm{jG}$	$26.1 \pm 2ijklG$	$49.6\pm0.7\mathrm{iF}$

At -80 °C, similar to -20 °C, the Arianna and Silvia WF had a decreasing trend for vitamin C during storage, but presented a higher value than at -20 °C; the Francesca WF showed an increasing trend for vitamin C until the end of storage, except at 5 M. The DF also presented higher vitamin C values (with some exceptions) at -80 °C until the end of storage for all cultivars compared to -20 °C, but with dramatically lower values than the WF. There was a significant difference in fruit vitamin C content between fruits stored at -20 °C and -80 °C during storage and between treatments.

Ancos et al. [25] reported that vitamin C was highly preserved after freezing fruits, but the degradation increased during storage, confirming some of our results. The chemical changes that may occur after the freezing of berries, as a result of oxidation and enzymatic activity, might influence the degradation and loss of vitamin C content [26]. The concentration of reagents in the non-frozen phase of frozen fruits and crystallization can favor chemical and enzymatic oxidation reactions [27]. Regarding the drying process, vitamin C is highly sensitive to and unstable in heat, so it was degraded during the sample preparation and oven-drying process. Vitamin C is also hydrosoluble and, as 90% of strawberries are water, the loss of vitamin C occurs during the drying process [28].

#### 3.2. Phenolic Acids

Phenolic acids are a part of the large group of phenolic compounds, widely distributed in strawberry fruits. They are considered important ingredient of strawberries, contributing to taste, color, and nutritional properties [29].

The phenolic acids' content in the WF of all three cultivars during storage is shown in Table 4. The Arianna, Francesca, and Silvia fruits had a content of 396.4 mg/kg FW, 569 mg/kg FW, and 170 mg/kg FW phenolic acids at time 0, respectively. The oven-drying process reduced the amounts of phenolic acids in the fruits at time 0 to 192.6 mg/kg FW, 288.1 mg/kg FW, and 138.2 mg/kg FW, respectively.

**Table 4.** Average of phenolic acids' content (mg/kg FW) in cultivars of Arianna, Francesca, and Silvia fruits. The data are expressed as mean  $\pm$  standard error. Different lowercase letters indicate significant differences for  $p \le 0.05$  in each cultivar during storage. Different uppercase letters indicate significant differences for  $p \le 0.05$  for all cultivars for a specific storage time. WF: whole fruit, DF: dried fruit. 0 M: 0 months of storage; 1 M: 1 month of storage; 3 M: 3 months of storage; 5 M: 5 months of storage.

Storage Time	Storage Temperature	Treatment	Arianna	Francesca	Silvia
		WF	$396.4 \pm 15.3 \mathrm{abB}$	$569 \pm 1.9$ cA	$170.7 \pm 9.7 dE$
0 M		DF	$192.6\pm1 efD$	$288.1\pm4.3\text{gC}$	$138.2\pm1.3 fghF$
	-20	WF	$305.2 \pm 7.5 \mathrm{cB}$	$643.2\pm14.6\mathrm{aA}$	$206.9\pm3.3\text{bEF}$
1.) (	-80	WF	$273.4\pm5.4\mathrm{cC}$	$631.7\pm11.5\mathrm{aA}$	$222.5\pm1.9 \mathrm{aDE}$
1 M	-20	DF	$188.2 \pm 2.2 \mathrm{efF}$	$313.2 \pm 9.5 efgB$	$129.2\pm0.1$ ghijk $ m G$
	-80	DF	$201.2\pm2.3deEF$	$237 \pm 9.5 hD$	$131.2\pm 6.3$ ghijG
	-20	WF	$427.2\pm6.6aC$	$601.2\pm25.6\text{bB}$	$191.5 \pm 2.5 \mathrm{cEF}$
2.14	-80	WF	$400.2\pm6.6abC$	$646.8\pm14aA$	$191.6 \pm 5.5 \mathrm{cEF}$
3 M	-20	DF	$230.6\pm23.7 dE$	$386.2 \pm 5.3 \mathrm{dC}$	$164 \pm 12.8 deFG$
	-80	DF	$387 \pm 11.7 \mathrm{bC}$	$292.2\pm8.9 fgD$	$140.9\pm2.2 fgG$
	-20	WF	$162 \pm 3 \mathrm{fgE}$	$321.4 \pm 4.8 \mathrm{efA}$	$149.8 \pm 3.3 \mathrm{efE}$
	-80	WF	$191.5 \pm 4.4$ efC	$325.5\pm2.8 eA$	$129.7\pm2.8$ ghijkF
5 M	-20	DF	$91.4 \pm 0 hG$	$177.1 \pm 2.9$ jD	$123.1 \pm 4.7$ hijkF
	-80	DF	$151.8\pm10.4\mathrm{gE}$	$226.5\pm2hiB$	$117.5 \pm 2.3$ jkF
	-20	WF	$190.9 \pm 6.1 \mathrm{efB}$	$294.3\pm5 \mathrm{fgA}$	$134.5 \pm 2.4$ ghiCDE
7 ) (	-80	WF	$186.8 \pm 5.7 \mathrm{efB}$	$291.6 \pm 7 \mathrm{gA}$	$126.1 \pm 1.6$ ghijkDEF
7 M	-20	DF	$138.9 \pm 4$ gCD	$198.7\pm2.4$ ijB	$122.3 \pm 4.9$ ijkEF
	-80	DF	$144 \pm 3.3$ gC	$191.6\pm5.2 \mathrm{jB}$	$114.6 \pm 2.3$ kF

The phenolic acids' content in Francesca and Silvia WF increased at 1M during storage at -20 °C (643 mg/kg FW and 206.9 mg/kg FW, respectively) and had a decreasing trend until the end of storage. Differently, in Arianna, the highest amount was detected after 3 M of storage at -20 °C, with the value of 427.2 mg/kg FW. Regarding the DF, there was a decreasing trend until the end of storage, with a peak of phenolic acid content in fruits after 3 M of storage in all the cultivars.

At -80 °C storage temperature, the WF showed a similar reaction to -20 °C, with increased phenolic acids' content at the early stage of storage, then a decreasing trend until the end of storage. The highest amounts of phenolic acids were detected in Francesca fruit at 3M of storage, with the value of 646 mg/kg FW. Regarding the DF, the phenolic content of Francesca and Silvia fruits increased after 1M of storage, decreasing until the end of storage; Arianna showed a slightly different behavior, with increased phenolic acids' content from 1 M to 3 M, then decreasing until the end of storage, but there was not a significant difference between the different temperatures during storage, but there was a significant difference between the treatments in all the cultivars, with the WF phenolic acids' content always being higher than those of the DF. The same effect was observed in a storage study of frozen strawberries by Oszmainski et al. [30]. They reported that the degradation of phenolic acids may be due to enzymatic oxidation. The degradation of phenolic acids during the oven-drying process were also confirmed in studies by Coklar et al. [31] and Semenov et al. [32]. The oxidative and thermal degradation of phenolic compounds due to

the increasing heat treatment led to decreases in the phenolic compounds in oven-dried fruits [33].

## 3.3. Anthocyanins

Anthocyanins are the most important phenolic compounds of the strawberry [34], and their concentration increases during the ripening progress [35]. The Arianna, Francesca, and Silvia WF had a content of 202.2 mg/kg FW, 108.8 mg/kg FW, and 300.7 mg/kg FW of total anthocyanins at time 0, respectively (Table 5). The oven-drying process greatly reduced these amounts to 98 mg/kg FW, 77.4 mg/kg FW, and 108.6 mg/kg FW, respectively.

**Table 5.** Average of total anthocyanins' content (mg/kg FW) in cultivars of Arianna, Francesca, and Silvia fruits. The data are expressed as mean  $\pm$  standard error. Different lowercase letters indicate significant differences for  $p \le 0.05$  in each cultivar during storage. Different uppercase letters indicate significant differences for  $p \le 0.05$  for all cultivars for a specific storage time. WF: whole fruits, DF: dried fruits. 0 M: 0 months of storage; 1 M: 1 month of storage; 3 M: 3 months of storage; 5 M: 5 months of storage; 7 M: 7 months of storage.

Storage Time	Storage Temperature	Treatment	Arianna	Francesca	Silvia
0.14		WF	$202.4\pm2.7\mathrm{Eb}$	$108.8\pm5.5\mathrm{Cc}$	$300.7\pm3.3$ Ba
0 M		DF	$98 \pm 1.8$ Fd	$77.4 \pm 1.2 \mathrm{Ee}$	$108.6\pm0.5 Hc$
	-20	WF	$236.9 \pm 1.1 \text{abcC}$	$127.3 \pm 1.8 \mathrm{abD}$	$266.6\pm9.1\text{Cb}$
1 \ /	-80	WF	$224.7\pm14.4\mathrm{Dc}$	$123.3\pm1.1\text{Bde}$	$300.7\pm7.5\text{Ba}$
1 M	-20	DF	$61.1 \pm 0.8$ Gfg	$52.9 \pm 1.5$ Jg	$122.1\pm0$ Gde
	-80	DF	$50.8\pm0.7 \mathrm{ghG}$	$74.9 \pm 1.5 efF$	$109.4\pm3.8\text{He}$
	-20	WF	$249\pm0.1\mathrm{Aa}$	$101.4\pm0.6\mathrm{Cf}$	$187.6\pm0.4\mathrm{Fd}$
2 14	-80	WF	$244 \pm 1.2 abB$	$89.4\pm2.6 \mathrm{Dg}$	$207.4 \pm 1$ Ec
3 M	-20	DF	$45.8\pm0.8$ Hj	$61.2 \pm 0.6 hiH$	$111.9 \pm 1.1$ ghE
	-80	DF	$56.4 \pm 1.5 \mathrm{ghI}$	$64.8\pm0.4 ghH$	$92.1 \pm 1.9 \mathrm{Ig}$
	-20	WF	$203.6\pm2.9\mathrm{Ec}$	$132.7\pm0.3\text{Ad}$	$259.4 \pm 4.8 \text{Cb}$
	-80	WF	$200.1\pm5.7\mathrm{Ec}$	$108.9\pm6.3\mathrm{Ce}$	$335\pm2.6\mathrm{Aa}$
5 M	-20	DF	$19.5\pm0\mathrm{Ig}$	$69.2 \pm 1.4$ fgI	$72.1 \pm 0.8$ klG
	-80	DF	$56.1 \pm 1.2$ ghH	$57.1 \pm 0.5$ ijH	$86.6 \pm 1 \mathrm{ijF}$
	-20	WF	$232.1\pm2bcdB$	$124 \pm 1.5 \text{Bd}$	$298.7\pm2.6\text{Ba}$
7 14	-80	WF	$224.9\pm3cdC$	$126.3\pm4.1abD$	$220.2\pm1.4\text{Dc}$
7 M	-20	DF	$48.1 \pm 1.1 \mathrm{Hgh}$	$49.6\pm0.2$ Jgh	$77.8 \pm 2.8$ jkE
	-80	DF	$44.7\pm0.8Hh$	$51.1 \pm 0.6 Jg$	$62 \pm 1 Lf$

Storage duration and temperature do not significantly affect the anthocyanins amount in the WF. There was a slight increase in the amounts of the anthocyanins in the Arianna and Francesca WF from 1 month to 7 months, while in Silvia fruit the anthocyanins amount was maintained during storage. Regarding the oven-dried fruits, the anthocyanin content of Arianna and Silvia fruits dramatically decreased after 1 month, and the values remained very low until 7 months of storage; however, in Silvia fruit, the anthocyanin content increased after the first month of storage, and, by increasing storage time, the anthocyanin content decreased in parallel. The lowest amount of anthocyanin was found in the Arianna DF after 5 months of storage at -20 °C. There was also no significant effect of storage temperature on the anthocyanins' content of the dried fruit of all cultivars.

Freezing is commonly regarded as a technique that has a less deleterious effect on the anthocyanins of strawberries for long-term storage [36]. Kamiloglu [9] studied the effect of different freezing methods on the bioaccessibility of strawberry polyphenols and concluded that freezing retains the bioactive compounds of strawberries and might enhance the total amounts of bioaccessible anthocyanins. Freezing rate and storage time are the most important parameters in the losses of anthocyanin in strawberries during the freezing process. It has been determined that a higher freezing rate is essential for the better preservation of the bioactive compounds in strawberries. Freezing should be done at an appropriate freezing rate to preserve the cell structure and the nutritional content of strawberries [37]. The effects of heat on microorganisms and the activity of enzymes are important when drying fruits [15]. Some important properties of fruits change during dehydration such as texture, chemical changes affecting flavor and nutrients, and color, as the evolution of the color is strongly correlated with the anthocyanin concentration across various fruits and vegetables [38–40]. A high temperature during the drying process is an important factor for the loss of quality. Lowering the process temperature has great potential to improve the quality of dried products [41].

## 3.4. Folate

The folate content of all three cultivars during storage is shown in Table 6. The folate content of the Arianna, Francesca, and Silvia WF at time 0 was 126  $\mu$ g/kg FW, 171.55  $\mu$ g/kg FW, and 113.72  $\mu$ g/kg FW, respectively. The oven-drying process slightly decreased the folate content of the fruits of all cultivars, with the folate content being 120.96  $\mu$ g/kg FW, 115.19  $\mu$ g/kg FW, and 103.09  $\mu$ g/kg FW in the DF at time 0, respectively. The folate content of the WF during storage at -20 °C had an increasing trend from 1M until the end of storage for all cultivars, and, at the end of storage, the folate contents of the Arianna, Francesca, and Silvia WF were detected to be 190.61  $\mu$ g/kg FW, 208.4  $\mu$ g/kg FW, and 132.06  $\mu$ g/kg FW, respectively. In the DF, similarly to the WF, the folate content increased with the extension of storage for all cultivars.

**Table 6.** Average of folate content ( $\mu g/kg$  FW) in cultivars of Arianna, Francesca, and Silvia fruits. The data are expressed as mean  $\pm$  standard error. Different lowercase letters indicate significant differences for  $p \le 0.05$  in each cultivar during storage. Different uppercase letters indicate significant differences for  $p \le 0.05$  for all cultivars for a specific storage time. WF: whole fruits, DF: dried fruits. 0 M: 0 months of storage; 1 M: 1 month of storage; 3 M: 3 months of storage; 5 M: 5 months of storage; 7 M: 7 months of storage.

Storage Time	Storage Temperature	Treatment	Arianna	Francesca	Silvia
		WF	$126.17\pm4.8 \text{cdB}$	$171.55 \pm 1.39$ abcdA	$113.72\pm0.79 cdD$
0 M		DF	$120.96\pm2.73\text{dC}$	$115.19\pm1.27\text{dD}$	$103.09\pm1.78\text{dE}$
	-20	WF	$133.58\pm1.61 \text{cdB}$	$156.39 \pm 1.82 bcdA$	$114.88 \pm 1.44 cdCD$
1 ) (	-80	WF	$132.27\pm2.92 \text{cdB}$	$116.97\pm0.54\mathrm{dCD}$	$117.92 \pm 1.45 cdCD$
1 M	-20	DF	$112.37\pm0.88 \text{dDE}$	$131.47\pm3.77\mathrm{dB}$	$107.35\pm0.91 \text{cdEF}$
	-80	DF	$119.88\pm3.17\text{dC}$	$134.83\pm0.62\text{dB}$	$104.04\pm0.3\text{dF}$
	-20	WF	155.39 ± 2.85bcdC	$141.68\pm3.53cdD$	$126.53 \pm 1.7 bcdEF$
2 1 4	-80	WF	$229.67\pm0.9a\mathrm{A}$	$223.59\pm5.4\mathrm{aA}$	$123.39\pm1.08 cdEF$
3 M	-20	DF	$127.6 \pm 2.99$ cdE	$164.8 \pm 2.94 \mathrm{abcdB}$	$106.5 \pm 0.13$ cdG
	-80	DF	$139.74\pm2.94bcdD$	$119.27\pm1.52\text{dF}$	$110.13\pm0.54 cdG$
	-20	WF	$147.71 \pm 7.25 bcdCDE$	$220.68\pm3.37aA$	$121.9\pm24.31 cdEF$
<b>Г ) (</b>	-80	WF	$131.23 \pm 2.11 cdDEF$	$132.08\pm2.18 \text{dDEF}$	$177.65\pm5.68\mathrm{aBC}$
5 IM	-20	DF	$157.83 \pm 12.88$ bcdCD	$150.22 \pm 2.41$ bcdCDE	$121.43\pm15.66 \text{cdEF}$
	-80	DF	$188.49\pm7.18abB$	$109.92\pm5.94\text{dF}$	$138.88\pm8.78 bcDEF$
	-20	WF	$190.61\pm3.66abAB$	$208.4\pm7.24abA$	$132.06 \pm 1.2 bcdAB$
7 14	-80	WF	$175.58\pm7.32 bcAB$	$199.44\pm51.7 \mathrm{abcAB}$	$157.55\pm1.75abAB$
/ INI	-20	DF	$174.64\pm40.41 bcAB$	$156.41 \pm 54.41 \text{bcdAB}$	$124.8\pm32.44cdAB$
	-80	DF	$188.04\pm58.92abAB$	$166.46\pm38.51abcdAB$	$102.66\pm1.63\text{dB}$

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At -80 °C storage, the folate content of the WF for all cultivars increased until the end of storage, as registered for the -20 °C storage. The Arianna and Francesca fruits had a peak of folate content after 3M of storage, with a content of 229.67 µg/kg FW and 223.59 µg/kg FW respectively, while the Silvia fruit had a peak after 5M of storage, with a content of 177.65 µg/kg FW. In the DF, there was also an increasing trend for folate content until the end of storage, with the highest content detected at the end of storage in the Arianna and Francesca fruits (188.04 µg/kg FW and 166.46 µg/kg FW, respectively); in the Silvia fruit, the highest folate content was detected after 5M of storage, with a value of 138.88 µg/kg FW. There was not a significant difference in folate content between temperature and treatments, but the WF presented more folate content than the DF during storage.

The strawberry is one of the most important sources of antioxidants and is considered a functional fruit due to the presence of a diverse range of bioactive components and high levels of vitamin C, vitamin E, folate, phenolic compounds, and fiber [42]. In this respect, variations of the amount of vitamin C in strawberries can affect the folate content, as a higher vitamin C content can lead to increased stability of folate [43]. The retention of folate and ascorbic acid was affected by the same factors, and a high content of ascorbic acid could provide possible protection against folate degradation [44]. This assumption was also confirmed by Ringling and Rychlik [45], who performed in vivo studies to simulate food folate digestion and found out that ascorbic acid stabilizes folate, particularly 5-CH3-H4 folate during digestion. The addition of ascorbic acid in physiological amounts improved the stability of some types of folate, depending on the food matrix [45].

### 3.5. Correlation Matrix

The correlation matrix among the analyzed nutritional parameters indicated that there was a good positive correlation between anthocyanins and vitamin C content (0.70, p < 0.05). This means that these two classes of compounds presented a similar behavior in the tested conditions, decreasing dramatically with the oven-drying treatment, while they did not change greatly regarding storage temperature or length. Vitamin C also presented a medium correlation with phenolic acids (0.52) and a lower but still significant correlation with folate content (0.27) (Table 7).

	Anthocyanins	Vitamin C	Folate	Phenolic Acids
Anthocyanins	1.00	0.70 *	0.04	-0.03
Vitamin C	0.70 *	1.00	0.31 *	0.52 *
Folate	0.04	0.31 *	1.00	0.27 *
Phenolic acids	-0.03	0.52 *	0.27 *	1.00

**Table 7.** Correlation matrix among the nutritional parameters analyzed. \* indicates a significant correlation for  $p \le 0.05$ .

### 4. Conclusions

In this study, some interesting results were obtained, describing how the nutritional compounds of strawberries react during storage at different temperatures with different storage times and treatments.

- Higher amounts of nutritional compounds were detected in the WF compared to the DF.
- For preserving nutritional quality, the WF treatment was optimum, and it showed good results in -80 °C storage compared to -20 °C. This is an important indication for application in the laboratory analysis and processing industry.
- The anthocyanins' content in the WF seemed to not decrease during 7 months of -80 °C storage, with some exceptions.
- Oven drying was not an ideal treatment for preserving vitamin C, almost completely degrading this compound in strawberry fruits.

- In the WT and DF, the amount of folate increased during storage. More folate was detected in the WF.
- The different strawberry cultivars presented different amounts of nutritional compounds: the Arianna and Francesca fruits had more vitamin C, phenolic acids, and folates, but the Silvia fruit had more anthocyanins.
- At the end of storage (7M), there was more loss of vitamin C in the Arianna WF compared to the other two cultivars' WF.
- There was a slight increase in the amount of anthocyanin in the Arianna and Francesca WF after 7M of storage, but in the Silvia WF the amount of anthocyanin was retained.

Generally, it can be concluded that oven drying is not a recommended technique to treat fruits for preserving nutritional quality during storage. Storage time did not greatly affect the nutritional quality in whole fruits, though they presented a higher amount when stored at -80 °C.

**Author Contributions:** Conceptualization, L.M. and B.M.; methodology, R.Q.; software, R.Q.; validation, L.M., F.C. and B.M.; formal analysis, R.Q.; investigation, L.M. and B.M.; resources, B.M. and F.C.; data curation, L.M. and F.C.; writing—original draft preparation, R.Q.; writing—review and editing, L.M. and B.M.; visualization, R.Q.; supervision, B.M.; project administration, B.M.; funding acquisition, B.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Partnership for Research and Innovation in the Mediterranean Area (PRIMA)'s 2019–2022 MEDBERRY project.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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