Article

Preservation of Biologically Active Compounds and Nutritional Potential of Quick-Frozen Berry Fruits of the Genus *Rubus*

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Abstract: Cryoprotective freezing methods are increasingly being developed and used as an effective means of protecting valuable bioactive compounds in processed berry fruits. The quick-freezing method allows the bioactive compounds in the plant material to be preserved over a longer period of time, thus providing a high-quality product with significant antioxidant capacity. The aim of this study was to determine the effects of the quick-freezing method on physico-chemical properties and bioactive compounds content of fruits in three soft fruit species: tayberry, raspberry, and blackberry, and to evaluate the stability of specific phytochemicals during the three-month storage period. The freezing method had a significant effect on the physicochemical properties with a significantly less drip loss observed after thawing in fruit frozen by quick-freezing (at -34°C for 25 min) compared to fruit frozen classically (-18°C to 24 h). The color of quick-frozen fruits also changed significantly less compared to fresh fruits. Of the bioactive compounds analyzed, it should be noted that there was a significantly lower loss of ascorbic acid recorded during quick-freezing. On average, the quick-frozen fruits contained 28% more ascorbic acid than the classical frozen fruits. In general, the quick-freezing procedure contributed to a better preservation of total polyphenolic compounds and anthocyanins, and thus berry fruits also showed higher values of antioxidant capacity during quick freezing than during the classical procedure. During the storage period of three months, a decrease in the content of all the bioactive compounds studied was observed, although it should be emphasized that this loss during storage was not as pronounced in fruits frozen by the quick-freezing method as in classically frozen fruits. It can be concluded that the quick-freezing contributes significantly to the preservation of valuable bioactive compounds of berries and that this processing method can be considered important for maintaining the nutritional properties of berry fruits.

Keywords: berry fruits; phytochemicals; phenols; nutritional quality; antioxidant capacity; storage; freezing method

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1. Introduction

Water is one of the most important components of food and, as a key medium or environmental factor, is involved in the formation of almost all chemical, biological, and physical processes. After harvesting the raw material, water content is one of the most important limiting factors to maintain the quality of the raw material, i.e., the fresh food intended for consumption. Indeed, free moisture and numerous enzymes active in post-harvest processes can significantly affect the content of biologically active compounds such as phenols, vitamins, and other phytochemicals and, above all, cause their degradation, thus reducing the nutritional potential and the overall quality of the raw materials [1]. Berry fruits are characterized precisely by their favorable nutritional composition; they are rich

in a specialized metabolites or phytochemicals with strong antioxidant activity and thus have a very important beneficial effect on human health. Compounds such as polyphenols (flavonoids, phenolic acids, flavonols), anthocyanins (cyanidin, pelargonidin), quercetin, rutin, vitamins (vitamin C), and carotenoids (zeaxanthin, alpha-carotene, beta-carotene) have a strong positive influence on human health [2], and are abundant in berry fruits species, have anti-inflammatory, anti-allergic, and anti-cancer effects precisely because of the significant antioxidant action, i.e., inhibition of oxygen reactive species (ROS) in human cells, and are therefore effective in the prevention of many chronic diseases [2–7].

The berry fruits are characterized by a high-water content (as well as sugar) and require specific post-harvest conditions that result in slowing down the biochemical processes by enzymes and inhibit the growth of microorganisms, thus ensuring a longer shelf life and generally a longer product life. Precisely because of their high perishability and seasonality, the berry fruits except in fresh state, whose shelf life is relatively short, are often processed or subjected to processing operations that collectively remove large amounts of water, slowing and/or stopping undesirable processes that lead to the degradation of the nutritional quality of the product. One of the most efficient processes for the long-term preservation and storage of fruits, which has a minimal impact on the nutritional quality of the food, is freezing. In general, lowering the temperature below the freezing point of a raw material allows the inhibition of many metabolic processes in the fruit, slows down the growth kinetics of microorganisms, and ultimately prevents the degradation reactions of important phytochemicals. Main limiting factor of the freezing process, as a result of water mobilization by crystallization in the form of ice crystals, is a reduction in the amount of water, resulting in reduced product freshness, crystallization of amorphous solutes, which can lead to the degradation of some important bioactive compounds and mainly affect the deformation of the texture and structure of the product and deteriorate the characteristic color of the product. This is strongly influenced by the size and position of the ice crystals formed, which, depending on the type of freezing process applied, may result in greater or weaker damage of cell membranes and degradation of the physical structure, which ultimately manifests itself in reduced product quality after thawing [8,9]. On the other hand, some authors state [8–11] that certain freezing processes show positive effects on the functional properties of the raw material, as freezing can favor the release of bound compounds such as phenolic acids and anthocyanins and thus the release of bioactive compounds.

Precisely, because of all this, more and more cryoprotection methods are being developed, i.e., the use of much lower freezing temperatures (cryogenic), which make it possible to reach the freezing point of the raw material in a very rapid time, just a few seconds. In fact, the use of quick-freezing methods allows the formation and growth of smaller ice crystals in greater numbers, which ultimately do not cause excessive damage to the plant cell and, consequently, to other physical and chemical properties of the product, as is the case with classical freezing methods ($-18\text{ }^{\circ}\text{C}$, over 24 h). Depending on the refrigerant used (cryogenic gas), quick-freezing processes can last only a few seconds to a few minutes (e.g., using liquid nitrogen at temperatures down to $-196\text{ }^{\circ}\text{C}$ only a few seconds) [9,12–15]. It should also be emphasized that the authors point out that foods frozen using quick-freezing methods have a longer shelf life, i.e., their nutritional quality is not affected as much during the storage period as is the case with foods frozen using conventional methods [9,15–18]. Based on all the above, the aim of this study was to determine the effect of the quick-freezing method on bioactive compounds content in three berry fruit species: tayberry (*Rubus fruticosus* $\times$ *idaeus*), raspberry (*Rubus idaeus*), and blackberry (*Rubus fruticosus* agg.), and to evaluate the stability of specific phytochemicals during the storage period of the frozen fruits.

2. Materials and Methods

2.1. Plant Material

The following berry fruit species were conducted in the study: tayberry (*Rubus fruticosus* × *Rubus idaeus*), raspberry (*Rubus idaeus*) cv. ‘Tulameen’, and blackberry (*Rubus fruticosus* agg.) cv. ‘Loch Ness’. All berry fruit species were grown and harvested on the family farm in Koprivnica (Croatia) in July at the optimal time of ripening. The berry fruits were harvested by hand, in the early morning hours during dry weather. Only healthy fruits without visible signs of damage were randomly collected in PET containers, with a total weight of fruits per container of 125 g. A total of 7 PET containers were harvested for each berry fruit species. Immediately after harvesting, the fruits were transported to the Department of Agricultural Technology, Storage and Transport, Faculty of Agriculture, University of Zagreb, where they were stored in a refrigerator (4 °C, 87% humidity) until the planned freezing procedures (1 day). In addition, a part of the fruit was separated from each berry species in order to perform chemical analysis of the fresh fruit.

2.2. Freezing and Storage of Frozen Fruits

The fruits were first selected for freezing, while those with possible mechanical damage or visible signs of spoilage were removed. Only healthy fruits were then washed under a gentle stream of water. After washing, fruits were air-dried to remove excess water and separated for classical freezing (CLAS) and quick-freezing (QF). For the needs of classical freezing, berries were packed in PET plastic bags, about 20 fruits for each species in a total of 5 replicates. Classic freezing was performed at −18 °C for 24 h in a classic freezer (Lth 1, Gorenje, Ljubljana, Slovenia). For the purpose of quick-freezing, fruits were placed in plastic containers (average size 142 × 94 × 35 mm) with openings of 50 mm diameter, a total of 15 fruits per container in 5 replicates (each species had 5 replicants for freezing). The quick-freezing procedure was performed in a shock freezer (Tecnomac Sf 1, Castelfranco Veneto, Italy) at −34 °C for 25 min using carbon dioxide as refrigerant fluid (2200 g-R404A, GWP3922). The experimental design of berry fruits freezing procedures is shown in Table 1.

Table 1. The experimental plan for fruit-freezing of different berry species.

Species	Freezing Method	Temperature (°C)	Time (min)	Equipment	Refrigeration Fluid	Treatment
Tayberry	classical	−18	1440	classic freezer	R600a	Tay-CLAS
Tayberry	quick-freeze	−34	25	shock freezer	R404A	Tay-QF
Raspberry	classical	−18	1440	classic freezer	R600a	Rsp-CLAS
Raspberry	quick-freeze	−34	25	shock freezer	R404A	Rsp-QF
Blackberry	classical	−18	1440	classic freezer	R600a	Blc-CLAS
Blackberry	quick-freeze	−34	25	shock freezer	R404A	Blc-QF

Tay-CLAS—tayberry classical freezing; Tay-QF—tayberry quick-freezing; Rsp-CLAS—raspberry classical freezing; Rsp-QF—raspberry quick-freezing; Blc-CLAS—blackberry classical freezing; Blc-QF—blackberry quick-freezing.

The time required to completely freeze the fruits by classical freezing was 24 h (1440 min), while only 25 min was required in quick-freezing. Thawing was carried out at a temperature of 4 °C for both classically and quick-frozen fruits, and lasted for classic frozen fruits approximately 20 h, while for quick-frozen, it was 90 min. Storage of frozen fruits, both classic and quick-frozen, was carried out under the same conditions, i.e., at −18 °C for a total duration of 3 months.

2.3. Drip Loss Determination

To determine the loss of cell juice (water) during thawing, the fruits were weighed immediately after freezing (m1) and after thawing (m2) and calculated according to Equation (1) [19].

$$\text{Drip loss (\%)} = \frac{m1 - m2}{m1} \times 100 \quad (1)$$

2.4. Determination of Physico-Chemical Properties

All analyzes were performed on fresh berry fruits, while frozen fruits were thawed before analysis. For chemical analysis, both fresh and thawed berry fruits were homogenized and a uniform sample was prepared according to the procedure for each specific analysis. The physico-chemical parameters determined in berry fruits by standard laboratory procedures according to [20] were: total dry matter content (DM, %) by drying at 105 °C to constant mass, total soluble solids (TSS, %) by digital refractometer (Mettler Toledo, Refracto 30PX, Greifensee, Switzerland), total acidity (TA, %) by potentiometric titration, pH value by digital pH meter (Mettler Toledo, SevenMulti, Greifensee, Switzerland). For the purposes of color determination of fresh and frozen berry fruits, the total number of 30 fruits of each berry species were selected and by colorimeter (ColorTec PCM+, Meschede, Germany) according to the CIELAB method's chromaticity parameters (L^* , a^* , b^* , C , h°) were measured.

2.5. Determination of Specialized Metabolites Content

From the group of specialized metabolites, the following were determined: ascorbic acid (AsA) content by titration with 2,6-dichloroindophenol (DKF) according to [21]. AsA was isolated from fresh and frozen berry fruits by homogenizing 10 g $\pm$ 0.01 of the plant material with 100 mL of 2% (*v/v*) oxalic acid. The prepared solution was allowed to stand for about 20 min, filtered through Whatman filter paper, and the filtrate was obtained in a volume of 10 mL. The obtained 10 mL filtrate was titrated with freshly prepared 2,6-dichloroindophenol (DKF) until a pink color appeared. The final AsA content was calculated according to Equation (2) and expressed as mg/100 g fresh weight (fw) for samples in fresh state and as mg/100 g dry weight (dw) for frozen and stored samples.

$$\text{AsA (mg/100 g fw)} = \frac{V \text{ (mL)} \times F}{D} \times 100 \quad (2)$$

where, V —volume of DKF (mL); F —factor of DKF; D —sample mass used for titration.

From the group of polyphenolic compounds, total phenolics (TPC), total flavonoids (TFC), and total non-flavonoids (TNFC) were determined. The values of all the above polyphenolic compounds were determined spectrophotometrically (Shimadzu, UV 1650 PC) according to a method described by Ough and Amerine [22]. The method is based on a color reaction that phenols develop with Folin-Ciocalteu reagent, measured at 750 nm. The isolation of polyphenolic compounds (10 g $\pm$ 0.01 of plant material) was carried out with total of 100 mL of 80% EtOH (*v/v*) by heating to the boiling point with reflux for total time of 20 min. The extract thus prepared was used for the reaction with the reagent Folin-Ciocalteu as follows: 0.5 mL of ethanolic extract, 30 mL of distilled water (dH_2O), 2.5 mL of the prepared reagent Folin-Ciocalteu (1:2 with dH_2O), and 7.5 mL of saturated sodium carbonate solution (Na_2CO_3) were added; the flask (50 mL) was filled to the mark with dH_2O and the prepared sample was allowed to stand at room temperature for 2 h with intermittent shaking. The same ethanolic extracts prepared for TPC determination were used for TNFC content determination, while TNFC separation was performed according to the following procedure: 10 mL of the ethanolic extract was added to the 25 mL capacity volumetric flask, then 5 mL of HCl (1:4, *v/v*) and 5 mL of formaldehyde were added. The prepared samples were blown with nitrogen (N_2) and left for 24 h at room temperature in a dark place. After 24 h, the same Folin-Ciocalteu reaction was performed as for TPC. The absorbance of blue color in both TPC and TNFC reactions was measured spectrophotometrically at 750 nm using distilled water as a blank. Gallic acid and catechin were used as external standards and the concentration of TPC and TNFC content was expressed as mg GAE/100 g fresh weight (fw) and for the frozen and stored samples as mg GAE/100 mg dry weight (dw). The TFC content was mathematically expressed as the difference between total phenols and non-flavonoids. Total anthocyanin content (TAC) was determined according to the bisulfite bleaching method described by Ough and Amerine [22]. Plant material (2 g $\pm$ 0.01) was weighed into a cuvette volume of 50 mL, to

which 2 mL of 0.1% HCl (diluted with 96% EtOH) and 40 mL of 2% HCl (*v/v*) were added. The prepared samples were centrifuged at 4500 rpm for 10 min. After centrifugation, 10 mL of the supernatant was separated into one test tube and 10 mL into another test tube. To the first test tube, 4 mL of dH₂O was added (blank), while to the second test tube, 4 mL of a freshly prepared solution of 15% sodium bisulfite (NaHSO₃, *v/v*) was added. The samples thus prepared were allowed to stand in the test tubes for 15 min, while thereafter the absorbances (A1 for the blank and A2 for the reaction with bisulfite) were measured spectrophotometrically at 520 nm using 2% HCl (*v/v*) as a blank. The final anthocyanin content (TAC) was calculated from the difference in absorbance (A1–A2) multiplied by the molar mass of the most abundant anthocyanin and expressed as mg/kg fresh weight (fw) and for the frozen and stored samples as mg/kg dry weight (dw).

2.6. Determination of Antioxidant Capacity

The antioxidant capacity assay was performed with an ABTS radical cation (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) and determined spectrophotometrically according to the method described by Miller et al. [23]. The same ethanolic extracts were used as for the polyphenolic compounds. The Trolox was used as antioxidant standard (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich, St. Louis, MO, USA) both for the preparation of the stock solution and appropriate dilutions to create the calibration curve. 5 mL of ABTS solution (7 mM) and 88 mL of potassium persulfate solution (140 mM) were mixed and left in the dark at room temperature for 16 h to obtain an ABTS radical (ABTS●1). On the day of analysis, a 1% ABTS●1 solution (in 96% EtOH) was prepared. A total of 160 µL of the extract was directly injected into the cuvette and mixed with 2 mL of stable ABTS●1 and the absorbance was measured at 734 nm using 96% EtOH as a blank. Final results were expressed as µmol TE/L.

2.7. Statistical Analysis

The data obtained were analyzed using SAS® version 9.3 [24]. One-way analysis ANOVA was used to determine the significance of differences within the berry species in fresh state. Freezing treatments were arranged in a randomized complete block design and all laboratory analyzes were performed in triplicate. A generalized linear model (PROC GLM) with replicates and interactions of berry species (tayberry, raspberry, blackberry), freezing method (classic, quick-freezing) and storage was used for the analysis. In both analyzes, means were compared using *t*-test (Least Significant Difference, LSD) and considered significantly different at $p \leq 0.01$. In addition to the results, the tables show different letters, indicating significant statistical differences between the different treatments at $p \leq 0.0001$. The average deviation of the results from the mean for each parameter studied with the standard deviation values are also indicated.

3. Results and Discussion

3.1. Physico-Chemical Properties, Bioactive Compounds Content, and Antioxidant Capacity of Fresh Berry Species

Berries are popular fruits mainly because of their excellent taste and aroma, but also because of their rich content of specialized metabolites, bioactive compounds, such as anthocyanins, flavonoids, phenolic acids, ellagitannins, etc. Due to their valuable nutritional composition and phytochemical content, berry fruits show numerous functional properties, ranging from antioxidant, antimicrobial, anti-inflammatory, to anticancer activities, thus strongly contributing to human health and representing a valuable nutritional source [2,25,26]. Due to specific physico-chemical parameters, berry fruits are often a valuable raw material for processing into various products such as juices, jams, jellies, syrups, dried fruits, etc., in addition to being eaten fresh. Tables 2 and 3 show the basic physico-chemical properties and bioactive compounds content of selected berry fruits in the fresh state. Highly significant statistical differences were found between different berry species for all analyzed physico-chemical parameters. Berry fruits are characterized by

high water content, therefore post-harvest procedures and manipulations from harvest to storage and marketing are often very difficult and demanding. Depending on the species and cultivar, the average water content of berry fruits is in the range of 80–85% [27,28], and generally depends mainly on pedoclimatic conditions, cultivation method, and agrotechnical practices, as well as genetic characteristics. According to the results within this study, the highest total dry matter content (DM) was found for both raspberry and blackberry (average 15.71%), while a slightly lower DM content was determined in tayberry fruit, about 8% lower compared to raspberry and blackberry. In addition to water content, berry fruits are also characterized by their high sugar and total acid content, the ratio of which is important for the formation of the final taste, which is why these fruits are so popular for fresh consumption. The total soluble solids content (TSS) was similar in all three species studied, the highest being found in blackberry (12.23%) and the lowest in tayberry (11.67%). The total acidity content (TA) varied in the range from 1.07% in blackberry to 1.73% in raspberry. Due to the relatively high TA content, low pH values were expected. The average pH value obtained in the analyzed berry species was 3.13, with the lowest value recorded in raspberry, the species with the highest TA value, while the highest pH value was recorded in tayberry. The obtained values of the analyzed physico-chemical parameters of the studied berry species are generally in agreement with other literature citations [27–29] with a deviation in the TSS content of tayberry. Pantelidis [27] stated an average TSS value of 6%, which is a significant difference from the data obtained in this study (11.67%). The reason may be the factors already mentioned, primarily the pedoclimatic characteristics of the cultivation area, but also the agrotechnical practices applied during cultivation (fertilization, irrigation, pruning, etc.).

Table 2. Physico-chemical properties of fresh berry fruits.

Species	DM (%)	TSS (%)	TA (%)	pH
Tayberry	14.56 ^b ± 0.38	11.67 ^b ± 0.29	1.12 ^b ± 0.03	3.25 ^a ± 0.03
Raspberry	15.65 ^a ± 0.36	12 ^{ab} ± 0.1	1.73 ^a ± 0.006	3 ^c ± 0.001
Blackberry	15.77 ^a ± 0.39	12.23 ^a ± 0.29	1.07 ^c ± 0.02	3.14 ^b
ANOVA	$p \leq 0.0142$	$p \leq 0.0405$	$p \leq 0.0001$	$p \leq 0.0001$

DM—total dry matter content; TSS—total soluble solids content; TA—total acid content. Results are expressed as mean ± standard deviation. Different letters indicate significant differences between mean values.

Table 3. Chromatic parameters of fresh berry fruits.

Species	L*	a*	b*	C	h°
Tayberry	23.2 ^b ± 0.67	18.51 ^b ± 2.37	4.01 ^b ± 1.27	18.97 ^b ± 2.95	11.88 ^b ± 1.93
Raspberry	31.35 ^a ± 0.62	26.32 ^a ± 0.88	8.59 ^a ± 0.76	27.69 ^a ± 1.02	17.89 ^a ± 1.13
Blackberry	20.19 ^c ± 0.16	1.35 ^c ± 0.15	0.39 ^c ± 0.06	1.41 ^c ± 0.15	15.87 ^a ± 2.36
ANOVA	$p \leq 0.0001$	$p \leq 0.0001$	$p \leq 0.0001$	$p \leq 0.0001$	$p \leq 0.0203$

Results are expressed as mean ± standard deviation. Different letters indicate significant differences between mean values.

Table 3 shows the color parameters of fresh selected berry fruits. According to the Hunterlab [30], an L* value indicates the intensity of light or darkness. When the value of L* = 0, there is no reflection, indicating the presence of black, and when L* = 100, the reflection is greatest, indicating the presence of white. A value of a* indicates the intensity of red or green, so negative values (−a*) indicate the presence of green, and positive values (+a*) indicate the presence of red. A value of b* indicates the intensity of yellow or blue, so negative values (−b*) indicate the presence of blue and positive values (+b*) indicate the presence of yellow. The parameters C and h° represent the color saturation, while the hue angle (h°) indicates the color saturation. Based on the obtained results, the lowest L* value (20.19) was obtained for blackberry, as expected, since blackberry is specific for its dark blue/purple coloration among the other two berry species analyzed, while for raspberry (specific light red/pink coloration of fresh fruits), the obtained L* value was the

highest (31.35). Since tayberry is a hybrid between raspberry and blackberry, the obtained L^* value (23.2) is between the obtained L^* values of raspberry and blackberry, as expected. Also, the obtained a^* and b^* values for all the examined berry species in this study are consistent with their specific coloration of the fresh fruits. For example, higher a^* and b^* values were obtained for raspberry (26.32; 8.59) and tayberry (18.51; 4.01) as an indicator of more red color, while significantly lower a^* values (1.35) were obtained for blackberry in conjunction with lower b^* values (0.39) as an indicator of more blue/purple color. All the obtained results of the analyzed chromaticity parameters are in agreement with other literature data [25,28], indicating that the selected berry fruits in this study were at an appropriate stage of maturity and achieved specific fruit coloration for each berry species. It is important to note that external color intensity can often be correlated with specific pigments synthesized in the fruit [31–33].

The most valuable compounds from the nutritional content of berry fruits are specific phytochemicals that position berries among the fruits with the highest antioxidant activity. Berries contain a considerable amount of various biologically active compounds, mainly ascorbic acid and phenolics (anthocyanins, flavanols, flavonoids, phenolic acids, tannins), which either singly or in combination have various functional properties that are essential for human health [2,5,34]. It is important to mention that numerous scientific studies prove that genetic traits, together with biotic, abiotic, and anthropogenic factors, have a significant influence on the synthesis pathways of metabolism and the accumulation, and thus the final content of specialized metabolites in berry fruits [35–37]. According to other scientific studies, the AsA content in raspberries is in the range of about 15–60 mg/100 g fw [26–29], while in this study, higher values were found. Then, by comparing the results of AsA in tayberries, higher values were also obtained in this study compared to other literature data that indicate values of about 20–60 mg/100 g fw [26–28]. As for the value of AsA in blackberries, values of 15–35 mg/100 g fw were found in other literature citations [26,27,29,37], while in this study significantly higher values were obtained, even about 2 times. The content of total phenolics (TPC), non-flavonoids (TNFC), and flavonoids (TFC) also differed greatly in the selected berry species (Table 4). The highest TPC value was determined in blackberry (512.93 mg GAE/100 g fw) and the lowest in raspberry (187.57 mg GAE/100 g fw). Due to the high TPC content, the values of TNFC and TFC are also relatively high; TNFC values range from 75.94 mg GAE/100 g fw (raspberry) to 251.58 mg GAE/100 g fw (blackberry), while TFC values range from 109.62 mg GAE/100 g fw (raspberry) to 261.34 mg GAE/100 g fw (blackberry). These results are also consistent with other literature data, which also state that the blackberry and tayberry generally have higher TPC values compared to raspberry [26–29,37]. According to other literature, TPC values in raspberry ranged from 200–700 mg GAE/100 g fw [26,28,29,37], in tayberry about 200 mg GAE/100 g fw [26], while in blackberry in the range of 250–600 mg GAE/100 g fw [26,29,37]. Genetic traits also had a significant effect on total anthocyanin content (TAC), as significant differences in TAC were observed among the berry species studied (Table 4). As expected, the highest TAC (1557.18 mg/kg) was determined in blackberry, being a species with characteristic dark blue/purple coloration, while the TAC value was as much as 2.5 times lower in tayberry (purple coloration) and as much as 7 times lower in raspberry (red/pink coloration). These results can be supported by other literature data, which also state that blackberries have higher TAC value compared to tayberries and especially raspberries [26–29,37]. Indeed, based on the obtained results of the content of phytochemicals in different berry species analyzed in the fresh state, it can be concluded that genetic characteristics have a significant influence on the content, but also comparing with other literature, it should be emphasized that besides genetic characteristics, pedoclimatic conditions during the cultivation of berry fruits also show a strong influence.

Table 4. Content of bioactive substances in fresh berry fruits.

Species	AsA (mg/100 g fw)	TPC (mg GAE/100 g fw)	TNFC (mg GAE/100 g fw)	TFC (mg GAE/100 g fw)	TAC (mg/kg)
Tayberry	128.34 ^b ± 3.64	330.22 ^b ± 0.29	152.81 ^b ± 0.59	177.41 ^b ± 0.57	606.8 ^b ± 4.26
Raspberry	70.94 ^c ± 2.84	185.57 ^c ± 0.46	75.94 ^c ± 0.52	109.62 ^c ± 0.75	221.61 ^c ± 1.94
Blackberry	142.46 ^a ± 2.49	512.92 ^a ± 1.42	251.58 ^a ± 1.03	261.34 ^a ± 1.05	1557.18 ^a ± 1.85
ANOVA	$p \leq 0.0001$	$p \leq 0.0001$	$p \leq 0.0001$	$p \leq 0.0001$	$p \leq 0.0001$

AsA—ascorbic acid content; TPC—total phenol content; TNFC—total non-flavonoid content; TFC—total flavonoid content; TAC—total anthocyanin content. Results are expressed as mean ± standard deviation. Different letters indicate significant differences between mean values.

Based on the high determined content of phytochemicals, the determined values of antioxidant capacity of the selected berry species were also high (Figure 1). The contribution of individual phytochemicals to the antioxidant activity of individual berry fruit species has been investigated in some studies [28]. For example, Beekwiler et al. [38] reported a relatively low contribution of vitamin C to the antioxidant activity in raspberries, about 20%, while for anthocyanins, about 25% of the antioxidant capacity of red raspberry fruits was reported. Poiana et al. [17] and Viskelis et al. [28] found highly correlated antioxidant activity with total phenolics content. According to the obtained results, the analyzed berry species differ significantly in terms of antioxidant capacity, the highest being found for blackberry (1764 µmol TE/kg) and the lowest for raspberry (1742 µmol TE/L). These results were expected based on the determined content of phytochemicals, as blackberry stands out with the highest content of vitamin C, TPC (TNFC, TFC), and TAC.

Figure 1. Antioxidant capacity (µmol TE/L) of berry fruit species in fresh state. Ant_cap—antioxidant capacity. Different letters indicate significant differences between mean values at $p \leq 0.0001$.

3.2. Drip Loss of Frozen Berry Fruits

One of the greatest disadvantages of the freezing process, generally for all fruits and vegetables, but especially for soft fruits (because of their high-water content), is thawing, or the loss of fruit juice during thawing (drip loss). Thawed fruit inevitably changes texture—the tissue becomes softer, which is ultimately also an undesirable characteristic for the consumer. The change in texture and, above all, the greater loss of cell juice is more pronounced when using classical methods of freezing (temperatures around -18°C and duration 24 h) mainly because such conditions mean slower freezing rate, during which larger ice crystals form in the cell tissue, causing considerable damage to cells and tissue. Upon thawing, these large crystals result in a higher release of water, which, as mentioned earlier, results in undesirable changes in the texture of the product. In contrast,

quick freezing at lower freezing temperatures (approximately -30 to -150 °C) and much shorter time (approximately 30 min or shorter) results in the formation of a larger number of small ice crystals and thus fewer changes in tissue texture, which is considered one of the most valuable advantages of the quick-freezing process [15]. In this study, we monitor the drip loss, cell sap loss, during thawing of different berry species with respect to the freezing method, and during storage of three months (Figure 2). Comparing the berry species regardless of the freezing method, blackberry shows the lowest average drip loss during thawing (average 1.84%), while tayberry shows the highest (3.44%). These results are expected due to the characteristic morphological fruit structure of each berry species; blackberry fruits are characterized by a higher, more pronounced fruit firmness, while raspberry and tayberry have a much lower fruit firmness (softer tissue) and a thinner fruit skin. If we consider the freezing method, regardless of the berry species, significantly higher values of drip loss are obtained with classic freezing, on average 4.08%, which even means a four-fold higher loss compared to quick-freezing (on average 0.95%). It is also important to emphasize that water loss significantly affects the thawing process itself. It should be noted that when the thawing rate is higher, the water loss from the fruit is less pronounced [14]. And since classically frozen fruit typically takes about 24 h to thaw, while quick frozen fruit takes about 30 min, it is expected that water loss will be significantly less in quick frozen fruit. During the three-month storage period (Figure 2), a slightly lower drip loss was observed, but again significant differences were found in relation to the freezing method, with significant drip loss observed in classically frozen fruits, on average 1.54%, irrespective of berry species, while in quick frozen fruits, this loss was slightly lower, on average 1.1%. Also, significant differences of drip loss during storage were observed depending on the berry species, but now during storage, a higher drip loss was observed for blackberry fruit, on average 1.63%, while the lowest was observed for tayberry fruit (on average 1%).

Figure 2. Drip loss of frozen berry fruits and after storage as a function of freezing method. Different letters indicate significant differences between mean values at $p \leq 0.0001$. Significance of interaction: $V \times F$ —variety and freezing method $p \leq 0.2789$; $V \times S$ —variety and storage $p \leq 0.1531$; $F \times S$ —freezing method and storage $p \leq 0.0001$; $V \times F \times S$ —variety, freezing method and storage $p \leq 0.0001$.

3.3. Physico-Chemical Properties of Frozen Berry Fruits

One of the major challenges of the freezing process is the control and regulation of ice crystal growth, as the crystallization process causes structural changes, potential tissue damage, but also changes in nutrient composition and degradation of valuable bioactive compounds. It is for these reasons that the right choice of freezing method can successfully influence the quality of the final product. Many studies prove the advantages of using modern freezing methods (use of cryogenics, IQF method, etc.) in preserving the quality of

the final product [1,8,9,17,39]. However, above all, it is important to optimize the choice of the method itself, i.e., to adapt it to the type of fruit and the properties and conditions that significantly affect the preservation of each nutrient. Some phytochemicals show higher resistance, stability to certain processing conditions, such as temperature conditions, process duration, influence of the acidity of the medium, oxygen or light, etc.

The analyzed physico-chemical properties of frozen berry fruits are presented in Table 5. Significant differences of physico-chemical parameters were determined between all berry fruits depending on the freezing method (classical freezing, quick freezing) and storage. Total dry matter content (DM) values, regardless of berry fruit type, were generally higher in fruits that were classically frozen, with an average value of 16.44% compared to the quick-frozen fruits, where the average DM value was 15.38%. Thus, the water content in classically frozen fruits was slightly lower (average 84%) than in the quick-frozen fruits (average 85%), which is to be expected based on the obtained results of drip loss. Indeed, the drip loss was more pronounced in classically frozen berry fruits compared to the quick-frozen ones, so it can be concluded that the application of the quick-freezing process does not significantly affect the loss of water or cell sap during thawing. A slightly lower DM value (average 16.25%) was determined after 3 months of storage, but compared to DM after classical freezing, this is a reduction of about 1%, which is negligible and also depending on the statistical analysis, not significant. On the contrary, during the three-month storage period of quick-frozen berry fruits, regardless of the species, a slightly higher average DM value was observed, compared to the samples which were not stored; however, this increase is negligible (around 0.5%). On comparing the DM values in fruits after a three-month storage period depending on the freezing method, it is observed that slightly higher DM values (on average 16.25%) were determined in classically frozen berries, about 5% higher, which is in agreement with the obtained drip loss results. Therefore, after a three-months storage period, berries frozen by quick-freeze method generally had higher water content (on average 84.57%) compared to the classically frozen and stored fruits, which had on average 83.75% water content. These results are to be expected since we discussed above how the application of quick-freezing in fruits does not lead to such significant fractures and degradation of cell structures due to the formation of ice crystals, thus there was no significant water loss from the fruits during thawing. In conclusion, it can be stated that berry fruits frozen by the quick-freezing method do not show such a pronounced loss of water after three months of storage as fruits frozen by the classical method.

Total soluble solids content (TSS) was generally higher in berry fruits classically frozen—the average determined value, regardless of the berry species, was 11.93%, which is compared to the quick-frozen fruits: about 10% higher content. Depending on the berry species, a slight decrease in TSS was observed by quick-freezing in all the berries studied. During storage, a further slight downward trend for TSS content was observed in the classically frozen fruits (on average a decrease of 4%), but this trend was opposite in the quick-frozen fruits, namely a slight increase of TSS during storage of the quick-frozen fruits was observed. Sugars act as natural cryoprotectors (colligative type), thus reducing drip loss, preserving the original color and flavor of the fruit, and allowing higher retention of bioactive compounds in the plant material. The main cryoprotectants mechanism of sugars is based on the reduction of free water activity in cells, leading to a lowering of the freezing point; however, depending on their molecular weight, different sugar molecules also influence the ice formation process by modulating ice crystal growth. Monosaccharides, which are dominantly present in berry fruits, significantly lower the freezing point, allowing a significant amount of unfrozen water to remain even at very low temperatures [9,40]. Based on the above, it is expected that quick-frozen berry fruits generally have a lower total soluble solids content compared to classically frozen fruits. The organic acids of fruits are relatively stable compounds in relation to the freezing process, and in general their content is considerably preserved at lower temperatures. A slight decrease in the content of organic acids is often marked in long-term storage, as in the case of the TSS content, which generally depends on the rate of respiration of the fruit. But in

general, the respiration rate is significantly inhibited during storage at low temperatures, which allows the preservation of TSS and total organic acids [41,42].

Table 5. Physicochemical properties of frozen berry fruits.

Species	DM (%)	TSS (%)	TA (%)	pH
Classical freezing				
Tay-CLAS	15.37 ^{de} ± 0.12	11.50 ^b ± 0.1	1.39 ^h ± 0.01	3.19 ^b ± 0.02
Rsp-CLAS	17.61 ^a ± 0.27	12.53 ^a ± 0.06	1.86 ^d ± 0.02	2.96 ^{ef} ± 0.01
Blc-CLAS	16.33 ^c ± 0.18	11.77 ^b ± 0.15	1.16 ^j ± 0.05	3.08 ^c ± 0.01
3 months storage				
Tay-CLAS	15.74 ^d ± 0.18	10.93 ^{cde} ± 0.06	1.63 ^e ± 0.02	3.22 ^a ± 0.02
Rsp-CLAS	17.59 ^a ± 0.21	11.77 ^b ± 0.23	2.20 ^b ± 0.01	2.94 ^{fg} ± 0.01
Blc-CLAS	15.42 ^{de} ± 0.27	11.67 ^b ± 0.06	1.27 ⁱ ± 0.01	3.05 ^d ± 0.02
Quick-freezing				
Tay-QF	13.92 ^g ± 0.24	10.50 ^e ± 0.61	1.43 ^g ± 0.02	3.21 ^a ± 0.02
Rsp-QF	16.79 ^b ± 0.27	11.32 ^{bc} ± 0.5	2.02 ^c ± 0.03	2.97 ^e
Blc-QF	15.41 ^{de} ± 0.16	10.73 ^{de} ± 0.64	1.07 ^k	3.21 ^a ± 0.02
3 months storage				
Tay-QF	14.43 ^f ± 0.33	11 ^{cd}	1.58 ^f ± 0.03	3.17 ^b ± 0.01
Rsp-QF	16.65 ^{bc} ± 0.24	12.60 ^a ± 0.1	2.28 ^a ± 0.01	2.93 ^g ± 0.01
Blc-QF	15.23 ^e ± 0.14	11.53 ^b ± 0.12	1.26 ⁱ ± 0.01	3.19 ^b ± 0.01
ANOVA	$p \leq 0.0001$	$p \leq 0.0405$	$p \leq 0.0001$	$p \leq 0.0001$
V × F	0.0152	0.6167	0.2555	0.0001
V × S	0.1545	0.6690	0.0024	0.7868
F × S	0.7334	0.0014	0.9255	0.7381
V × F × S	0.0001	0.0001	0.0001	0.0001

DM—dry matter content; TSS—total soluble solids; TA—total acid content; V × F—interaction of variety and freezing method; V × S—interaction between variety and storage; F × S—interaction of freezing method and storage; V × F × S—interaction of variety, freezing method and storage. Results are expressed as mean ± standard deviation. Different letters indicate significant differences between mean values.

The total titratable acidity content (TA) of frozen berry fruits analyzed in this study is shown in Table 5. Significant differences were found, depending on both the freezing method, berry species, and storage. In general, TA was slightly lower (about 3% on average) in classically frozen berry fruits, regardless of berry species, compared to the quick-frozen fruits. But during the three-month storage period, there are significant changes in TA content, while the same increasing trend was observed for both classically and frozen fruits. Therefore, after the three-month storage period of classically frozen berry fruits, the TA content increased by 16% on average, while the TA content increased by about 13% for the quick-frozen fruits. This recorded increase in total organic acid content during storage differs from the data available in other studies, and a possible reason for this trend in this study may be a significant reduction in the respiration rate of fruits when processed and stored at lower temperatures, because the lower the storage temperature, the slower the respiration rate, which negatively affects the content of soluble substances, such as sugars or organic acids. For classically frozen berry fruits, the average pH was 3.08, while for quick-frozen, the average pH was 3.13, regardless of berry species (Table 5). During storage, a slight variation in pH was observed in both classic and quick-frozen fruits. The pH has a direct influence on fruit color, especially in berry fruits, as the anthocyanin content (and thus fruit coloration) is strongly influenced by pH of media. In addition, some studies have noted the high correlation between pH and color quality and calculated that berries should have a pH of 3.51 or lower to have acceptable color after freezing [43].

Color is one of the four main characteristics representing the quality of the final product, and it is one of the most valuable morphological parameters from a consumer's point of view. Fruit color is strongly influenced, not only by agrotechnical measures and agronomic factors during cultivation, but also by the different postharvest technological processes, such as pre-cooling, packaging, freezing, etc. [8]. The selection of an appropriate

method of berry processing is extremely challenging, mainly because of the characteristic soft fruit texture of these fruit species, each processing segment inevitably affects the final fruit quality, especially the color. Methods involving minimal processing and rapid freezing are one of the most desirable to preserve the original color of the fruit after freezing. Depending on the results of chromaticity parameters of different berry species studied in this research (Table 6), both the freezing method and the storage period have a significant ($p \leq 0.0001$) influence on fruit color. Significant changes in all analyzed color parameters were also observed between the fresh (Table 5) and frozen berry fruits studied. Namely, regardless of the freezing method, lower L^* values and higher a^* and b^* values were observed in frozen berry fruits, indicating a change in the characteristic berry color during the freezing process. In general, the fruits were darker after freezing and had a more pronounced red or blue coloration, depending on the species studied. Comparing the influence of freezing method (classical and quick-freezing) on fruit lightness/darkness (L^*) of all studied berry species, it can be observed that L^* values were lower after quick-freezing, indicating a darker fruit color; while a^* values (red coloration) did not differ significantly in tayberries and raspberries as a function of freezing method, except for blackberry, for which lower a^* values were observed in quick-freezing compared to classical freezing, indicating the lower presence of red coloration expected due to the specific dark blue/purple coloration of the fruits. The values of the chromaticity parameter b^* , indicating the presence of yellow ($+b^*$) and blue ($-b^*$) color, did not change significantly as a function of freezing method; a slight variation was observed in tayberry fruits for which a slightly lower b^* value was obtained after quick-freezing, indicating a greater presence of blue coloration. As expected, the storage period of three months also affected the color change of all berry species studied, but these differences were not so significant for both classically and quick-frozen fruits. In general, based on all color parameters analyzed, the color of quick-frozen berry fruits was slightly better preserved during storage for each specific berry species. However, since fruit color is directly related to the presence of specific pigments, in this case anthocyanins, the influence of freezing method and storage period on pigment compounds will be further clarified in the chapter on bioactive compound content.

Table 6. Chromatic parameters of frozen berry fruits.

Species	L^*	a^*	b^*	C	h°
Classical freezing					
Tay-CLAS	21.38 ^{dc} $\pm$ 0.96	23.37 ^b $\pm$ 0.79	5.14 ^{cd} $\pm$ 0.74	23.97 ^c $\pm$ 0.85	12.10 ^{cd} $\pm$ 1.69
Rsp-CLAS	28.93 ^b $\pm$ 2.62	30.84 ^a $\pm$ 0.73	11.26 ^a $\pm$ 0.29	32.96 ^{ab} $\pm$ 0.45	19.98 ^a $\pm$ 0.77
Blc-CLAS	19.59 ^{def} $\pm$ 0.59	10.24 ^c $\pm$ 0.82	2.11 ^e $\pm$ 0.19	10.46 ^d $\pm$ 0.85	11.98 ^{cd} $\pm$ 0.14
3 months storage					
Tay-CLAS	20.96 ^{cde} $\pm$ 1.14	23.41 ^b $\pm$ 1.49	4.89 ^c $\pm$ 0.99	23.94 ^c $\pm$ 1.66	11.59 ^{cde} $\pm$ 1.63
Rsp-CLAS	35.93 ^a $\pm$ 1.11	31.14 ^a $\pm$ 0.47	9.17 ^b $\pm$ 0.56	32.47 ^{ab} $\pm$ 0.60	16.39 ^b $\pm$ 0.72
Blc-CLAS	18.65 ^f $\pm$ 0.55	7.46 ^d $\pm$ 2.27	1.49 ^e $\pm$ 0.41	7.62 ^e $\pm$ 2.30	11.53 ^{cde} $\pm$ 2.58
Quick freezing					
Tay-QF	19.83 ^{def} $\pm$ 0.86	24.02 ^b $\pm$ 1.23	4.83 ^d $\pm$ 0.31	24.51 ^c $\pm$ 1.26	11.32 ^{cde} $\pm$ 0.17
Rsp-QF	28.73 ^b $\pm$ 2.93	31.30 ^a $\pm$ 1.04	11.69 ^a $\pm$ 1.10	33.39 ^a $\pm$ 1.35	20.35 ^a $\pm$ 1.25
Blc-QF	18.76 ^{ef} $\pm$ 0.33	8.40 ^{cd} $\pm$ 1.05	1.41 ^e $\pm$ 0.19	8.52 ^{de} $\pm$ 1.07	9.53 ^e $\pm$ 0.33
3 months storage					
Tay-QF	22.19 ^c $\pm$ 1.11	24.62 ^b $\pm$ 0.99	6.02 ^c $\pm$ 0.79	25.38 ^c $\pm$ 1.18	13.45 ^c $\pm$ 1.10
Rsp-QF	35.04 ^a $\pm$ 1.23	29.72 ^a $\pm$ 1.26	8.50 ^b $\pm$ 0.73	30.88 ^b $\pm$ 1.36	15.90 ^b $\pm$ 0.68
Blc-QF	18.61 ^f $\pm$ 0.15	10.06 ^c $\pm$ 1.21	2.01 ^e $\pm$ 0.19	10.27 ^d $\pm$ 1.22	11.17 ^{de} $\pm$ 1.63
ANOVA	$p \leq 0.0001$	$p \leq 0.0001$	$p \leq 0.0001$	$p \leq 0.0001$	$p \leq 0.0001$
V $\times$ F	0.9825	0.4378	0.8020	0.4361	0.4231
V $\times$ S	0.0001	0.6267	0.0001	0.2721	0.0001
F $\times$ S	0.8293	0.8752	0.8428	0.8692	0.6143
V $\times$ F $\times$ S	0.0002	0.0536	0.0001	0.0345	0.0011

V $\times$ F—interaction of variety and freezing method; V $\times$ S—interaction between variety and storage; F $\times$ S—interaction of freezing method and storage; V $\times$ F $\times$ S—interaction of variety, freezing method and storage. Results are expressed as mean $\pm$ standard deviation. Different letters indicate significant differences between mean values.

3.4. Bioactive Compounds Content and Antioxidant Capacity of Frozen Berry Fruits

The results of the content of bioactive compounds in frozen fruits of different berry species are presented in Table 7. According to the obtained results, all analyzed bioactive compounds differ significantly ($p \leq 0.0001$) according to the freezing method and storage period. In general, regardless of the berry species, values of AsA content were significantly higher in quick-frozen samples, compared to the classically frozen ones, with the mentioned increase being particularly pronounced in the quick-freezing of blackberries, where the AsA content was preserved to even 56%. It should be noted that the average AsA content in quick-frozen berry fruits, regardless of species, was as much as 28% higher, compared to the classically frozen fruits. Comparing the AsA content in frozen berry fruits, regardless of the freezing method, with the samples in fresh condition, a significant decrease in AsA content was observed, suggesting that freezing as a processing method also has a negative influence on AsA content. However, it should be emphasized that the mentioned decrease in AsA content was more pronounced in the classical freezing method, while by the quick-frozen method, the preservation of AsA content was more significant. As expected, the AsA content continued to decrease during the three-month storage period, regardless of the freezing method, but it should be noted that, in general, all berry species studied that were quick-frozen had higher AsA content after the storage period, on average about 13% higher, compared to the AsA content in classically frozen fruit. When comparing the effect of storage time in both classically and frozen fruits, it should also be noted that the decrease in AsA content was more pronounced in quick-frozen fruits; in general, these samples even had a 13% higher loss of AsA content during storage, compared to the classically frozen samples where only a small loss of AsA content was recorded during storage (less than 1%). The possible reason for such a variation in the loss of AsA content during storage of differently frozen berry species could be due to the storage conditions, as both the classically and frozen samples were stored at the same temperature ($-18\text{ }^{\circ}\text{C}$), which initially caused further formation and growth of larger crystals; respectively, recrystallization, which often occurs when the frozen product is subjected to large temperature fluctuations, and this affects the number, size, shape, and position of the ice crystals previously formed during freezing [44]. From the obtained results, it can be concluded that the use of the freezing system can significantly affect the total content of AsA, mainly affecting its degradation in the fruits of different berry species. According to other literature data, the degradation, i.e., the reduction in AsA content, is a necessary consequence of the freezing process and the storage period, regardless of the applied system, i.e., the freezing and cold storage conditions, temperature, and time [9,44]. Some of the authors state that the degradation of AsA after freezing could be associated with drip losses without involving the degradation of the compound itself, since water can act as a carrier agent [14]. In addition, most authors emphasize that the use of freezing methods that require faster freezing rates, such as quick-freezing methods, i.e., lower temperatures and shorter freezing times, can significantly contribute to the preservation of AsA content in berry fruits, compared to the classical slower freezing methods (temperatures around $-20\text{ }^{\circ}\text{C}$, 24 h) [9,14,45–47].

Table 7. Content of bioactive substances in frozen berry fruits.

Species	AsA (mg/100 g dw)	TPC (mg GAE/100 g dw)	TNFC (mg GAE/100 g dw)	TFC (mg CTH/100 g dw)	TAC (mg/kg dw)
Classical freezing					
Tay-CLAS	649.96 ^c ± 4.64	2275.26 ^g ± 2.69	1238.13 ^d ± 1.94	1037.38 ^d ± 9.41	771.42 ^d
Rsp-CLAS	623.32 ^c ± 2.84	1476.62 ^j ± 2.24	844.67 ^e ± 1.38	631.95 ^e ± 1.23	372.49 ^f
Blc-CLAS	614.77 ^c ± 7.34	3137.41 ^b ± 2.75	1691.99 ^a ± 1.25	1445.42 ^b ± 2.76	1537.30 ^a
3-month storage					
Tay-CLAS	649.43 ^c ± 4.86	2013.15 ^h ± 7.37	1505.19 ^b ± 1.7	507.95 ^g ± 2.82	727.75 ^d ± 4.78
Rsp-CLAS	607.89 ^c ± 1.98	1804.86 ⁱ ± 2.31	687.11 ^g ± 1.37	1117.75 ^{cd} ± 1.14	308.32 ^f
Blc-CLAS	621.68 ^c ± 2.04	2630.23 ^e ± 3.67	1386.65 ^c ± 4.46	1243.57 ^c ± 1.47	1420.45 ^d
Quick freezing					
Tay-QF	717.73 ^b ± 3.27	2469.45 ^f ± 1.05	1267.38 ^d ± 3.06	1202.08 ^c ± 2.65	839.89 ^c ± 2.84
Rsp-QF	733.52 ^b ± 2.29	1501.55 ^j ± 1.88	862.39 ^e ± 1.29	639.16 ^e ± 2.51	511.27 ^e
Blc-QF	956.08 ^a ± 3.31	3262.21 ^a ± 6.69	1712.01 ^a ± 3.37	1550.21 ^a ± 2.76	1560.87 ^a
3-month storage					
Tay-QF	781.28 ^a ± 5.25	2991.35 ^c ± 6.76	1523.40 ^b ± 3.62	1467.32 ^{ab} ± 3.05	820.11 ^d ± 1.55
Rsp-QF	745.29 ^b ± 2.81	1861.57 ⁱ ± 2.75	741.36 ^f ± 1.48	1120.21 ^{cd} ± 1.38	348.71 ^f
Blc-QF	598.24 ^c ± 3.13	2826.55 ^d ± 4.03	1407.05 ^c ± 1.27	1419.5 ^{ab} ± 1.52	1464.11 ^b
ANOVA	$p \leq 0.0001$	$p \leq 0.0405$	$p \leq 0.0001$	$p \leq 0.0001$	$p \leq 0.0001$
V × F	0.7220	0.0109	0.8691	0.0005	0.2787
V × S	0.0369	0.2170	0.0001	0.2398	0.2355
F × S	0.1008	0.6887	0.9835	0.3109	0.7623
V × F × S	0.0001	0.0001	0.0001	0.0001	0.0022

V × F—interaction of variety and freezing method; V × S—interaction between variety and storage; F × S—interaction of freezing method and storage; V × F × S—interaction of variety, freezing method and storage. Results are expressed as mean ± standard deviation. Different letters indicate significant differences between mean values.

Polyphenolic compounds are relatively not so prone to degradation by freezing process [48–50] and some authors even reported an increase in the phenolic compounds content after freezing [8,51]. Similar to those reported data, the results obtained in this study also indicate the increase in total phenol content (TPC) after freezing. Significant differences in all analyzed polyphenolic compounds were determined considering the freezing method (Table 7); in quick-frozen berry fruits, regardless of the species, on average 5% higher TPC content, 2% higher TNFC content, and 9% TFC content were recorded compared to the classically frozen berries. In general, the highest TPC, TNFC, and TFC values, both in quick-frozen and classically frozen samples, were determined in blackberry. Quick-freezing method most affected the preservation of TPC in tayberry species; approximately 9% higher TPC was recorded in quick-frozen samples, compared to the classically frozen ones. The storage period expectedly led to a further reduction in the content of total polyphenolic compounds, both in quick and classically frozen samples. After three-month storage in classically frozen berry fruits, regardless of the species, TPC content decreased about 7%, TNFC about 6%, and TFC content about 9%. During the storage of quick-frozen berries, some deviation in TPC content was recorded in tayberry and raspberry samples, wherein after storage, somewhat higher TPC values were determined. In blackberry, the same declining trend of TPC content in the storage period was recorded, both in quick and classically frozen fruits; in classically frozen blackberries about 19% lower TPC content was determined after three-month storage, while in quick-frozen, about 15% lower TPC was recorded after the storage period. One of the most characteristic compounds from the group of polyphenolics for the berry fruits are anthocyanins. As expected, the freezing method significantly affected ($p \leq 0.0001$) total anthocyanin content (TAC) of analyzed berry species (Table 7). In general, regardless of the berry species, the quick-freezing process contributed to better preservation of TAC. For instance, in quick-frozen blackberry fruits, about 2% higher TAC was recorded compared to the classical freezing, in tayberry fruits about 9% higher, and in raspberry even 37% higher TAC was determined. During the storage, further decrease of TAC was recorded in all analyzed berry species.

Approximately 10% loss of TAC was recorded both during storage of classically frozen and quick-frozen berries. The most pronounced loss of TAC during storage of classically frozen berry species—21%—was recorded for raspberries. Also, for the same species, a more pronounced loss (46%) of TAC was recorded during the storage of quick-frozen fruits. On comparing the TAC content of frozen berry fruits, it can be concluded that significant loss of TAC was recorded during freezing, with the exception of blackberry in which even higher TAC values were recorded specifically after quick-freezing. Structural tissue changes as well as disruption of cell integrity are a direct consequence of ice crystal formation during the freezing process, causing, among other things, further chemical and enzymatic changes leading to temporal reduction or loss of certain plant pigments (e.g., conversion of chlorophyll to phaeophytin), then browning of components due to enzymatic and oxidative reactions, and finally frequent loss of polyphenol and anthocyanin content [45,52]. Another possible cause for the degradation of polyphenolic compounds, especially anthocyanins, is the occurrence of leaching during the thawing process, where the compounds are more exposed to oxygen during the thawing phase and oxidation processes occur much faster [53]. It is also interesting to note that some studies (in addition to ours for some berry species) have found higher levels of polyphenols and anthocyanins after the freezing process. Indeed, an increase in content after freezing may lead to the release of binding phenolic compounds and anthocyanins [54,55].

As mentioned above, some authors reported an increase in the content of bioactive compounds (especially phenols and anthocyanins) after the freezing process, leading to an increase in antioxidant activity and consequently to an increase in the overall nutritional quality of the final product [8,10,51]. Freezing is recognized as a technology for the production of high-quality foods, as it has been shown to have very little effect on the reduction of bioactive compounds and consequently on the antioxidant properties of a product [9]. According to the results presented in Figure 3, the highest antioxidant capacity was recorded for blackberries in both quick-frozen and classically frozen berry fruits (quick-frozen 1911.07 $\mu\text{mol TE/L}$; classically frozen 1842.84 $\mu\text{mol TE/L}$). Both values are significantly higher than those recorded for blackberry in the fresh state (data from Figure 1). The greatest decrease in antioxidant capacity during freezing in general (regardless of the method) with respect to the berry type was determined for tayberry—about 2% compared to the fresh state. Considering that freezing can exert a negative effect on certain bioactive components (vitamins, phenols, and anthocyanins) due to cell rupture, which in turn leads to decompartmentalization of antioxidants, and their interaction with oxidative enzymes, ultimately leading to a decrease in their content, but also due to the freezing effect of the release of bound bioactive compounds, this can have a positive effect on the antioxidant properties of foods. Still, the results are contradictory according to different literature; it is important to mention that certain important factors ultimately affect the bioactive compounds and thus the antioxidant properties of the same: type of plant food, product structure, and type of process used. For example, some studies have shown that freezing of whole fruits has no or very little effect, compared to puree or juices [16,56–58]; others showed that liquid nitrogen freezing observed no change in the concentration of bioactive compounds, as well as other freezing methods that involve the use of lower temperatures in the shortest possible time [10,59]. As expected, during the storage period, a decrease in antioxidant capacity was observed in both the frozen and classically frozen samples. The most significant decrease during storage was observed in frozen blackberry fruits, about 10% in frozen samples and about 7% in classically frozen samples. The obtained results are as expected and in agreement with other literature data, where a significant decrease in antioxidant activity during storage time was found [17,60].

Figure 3. Antioxidant capacity ($\mu\text{mol TE/L}$) of frozen berry fruits and after storage, depending on the freezing method. Different letters indicate significant differences between mean values at $p \leq 0.0001$. Significance of interaction: $V \times F$ —variety and freezing method $p \leq 0.7530$; $V \times S$ —variety and storage $p \leq 0.0001$; $F \times S$ —freezing method and storage $p \leq 0.8837$; $V \times F \times S$ —variety, freezing method and storage $p \leq 0.0001$.

4. Conclusions

The studies carried out allow us to conclude that freezing, as a technological process, generally has a minor impact on the degradation of the content of bioactive substances (vitamins, phenols, anthocyanins) and effectively contributes to maintaining the quality of the final product. The selection of an appropriate freezing method is extremely important to maintain the nutritional quality of the product, and freezing temperature and duration are key factors in optimizing the appropriate method. The type of raw material to be frozen is also an important factor in process optimization, and ultimately the structure of the fruit (texture) has a significant influence on the selection of basic freezing parameters. The quick-freezing process, respectively lower freezing temperatures, and higher freezing rates ($-34\text{ }^{\circ}\text{C}$, 25 min) contributed significantly to the preservation of berry fruit phytochemicals, i.e., bioactive compounds: Ascorbic acid, total phenols, anthocyanins, and thus the total antioxidant capacity of berries compared to classical freezing ($-18\text{ }^{\circ}\text{C}$, 24 h). The external characteristics of the berry fruits, color and texture, changed less during freezing, focusing on the significantly lower drip loss after thawing and the color deterioration observed in the fruits frozen by the quick-freezing method, compared to the classical method. In general, it can be concluded that the quick-freezing method is extremely effective in preserving the nutritional quality of soft fruits. Future research should focus on more detailed analyzes of individual compounds using developed analytical methods (HPLC) in order to gain a deeper insight into the influence of freezing parameters on maintaining the quality of the final product.

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