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Oriental Strawberry Metabolites: LC–MS Profiling, Antioxidant Potential, and Postharvest Changes of *Fragaria orientalis* Fruits

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Abstract: *Fragaria orientalis* is the most distributed wild strawberry in the North Asia, and it is consumed in large quantities by the local population. However, poor knowledge of the chemical composition, metabolic profiles, and bioactivity leads to the insufficient use of *F. orientalis* beyond the region. The aim of our study was to investigate the nutritional value, chemical composition, and sensory parameters of wild and cultivated *F. orientalis* fruits, before and during postharvest storage. Sixty-five compounds were identified by liquid chromatography–mass spectrometry (LC–MS). Wild and cultivated fruits have the same LC–MS profiles, but quantitative levels varied, in favor of the wild samples. The extracts of *F. orientalis* fruit demonstrated high radical-scavenging potential, and ascorbic acid and various phenolics were identified as principal antioxidants. Significant variation in ascorbic acid, phenolic content, and total antioxidant potential was found during storage of the fresh *F. orientalis* fruit at cold and room temperatures and in defrosted fruits; storage of the dried fruits at cold temperature showed the lowest loss of bioactive compounds. The new information on postharvest changes in the phytochemicals and bioactivities provide a better understanding of the processes occurring in fresh, frozen, and dried strawberry fruits during storage.

Keywords: *Fragaria orientalis*; strawberry; phenolic compounds; ellagitannins; flavonoids; mass spectrometry; postharvest storage; antioxidant activity

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1. Introduction

Strawberry is the most popular and important soft fruit in the world, and its ever-growing global production increased by 40% in the last decade [1]. Numerous studies have established that strawberries are a rich storehouse of valuable substances, such as ascorbic acid, anthocyanins, ellagitannins [2], flavonoids [3], and antioxidants [4]. Despite the large production and consumption value, strawberry fruits are a very fragile food product, featuring short post-harvest storage time, owing to internal reasons (e.g., high respiration rate) and external reasons (e.g., handling or transportation lead to unwanted economic losses) [5]. The reduction of consumption properties is accompanied by a loss of phytocomponents, which are valuable compounds with confirmed biological activity [6]. Various processing methods (e.g., thawing or thermal treatment) can negatively influence the appearance, volatile composition, and polyphenol stability of strawberry pomace [7], causing a reduction of fruit quality. Thus, different methods of post-harvest storage are needed that would slow down the degradation process. Harvest conditions affect the polyphenol content in *F. ananassa*, such that higher fruit polyphenol content and anti-DPPH (2,2-diphenyl-1-picrylhydrazyl) activity have been observed during cold treatment, which suggests that strawberry fruits respond to cold stress immediately [8,9]. The application of

the cold storage of fresh strawberries saves more phytoconstituents and prevents the loss of bioactivity [10].

The garden strawberry (*F. ananassa*) is the most common *Fragaria* in the world and is used as an industrial strawberry; however, it is less adapted to harsh climatic conditions, which is the main problem for its widespread cultivation in Northern Asia. The gene pool of the genus *Fragaria* L. consists of dozens of species and subspecies, as well as numerous varieties [11]. Wild-growing strawberries significantly outperform *F. ananassa* varieties in winter hardiness; however, although most are possible sources of bioactive compounds and nutrients, they are not used in strawberry breeding, owing to the poor knowledge of phenotypic advantages and genetic potentials [12]. In recent years, research has been actively performed on the introduction of various types of wild strawberries into cultivation, especially *F. vesca*, which is a widespread forest strawberry in European and Central Eurasia [13]. This species has little distribution in the natural populations of Northern Asia, where it is replaced by an eastern strawberry or *F. orientalis* (Figure 1). Its western growing border passes through the Irkutsk region and extends to Buryatia (Lake Baikal) and to Yakutia up to 64° N and to Altai and the Far East. It grows in Mongolia, the northeastern provinces of China (Manchuria), and North Korea. It is an endemic of the relict mesophilic flora of the northeastern part of China [11]. *F. orientalis* is a rosette hemicryptophyte, and its above-ground part is laid off for the winter. The plant is a spring–summer green plant, according to the phenorhythmotype [14]. The oriental strawberry is an object of industrial gathering in Northern Asia, the harvesting volumes of which exceed those of the cultivated garden strawberries (*F. ananassa*) and green strawberries (*F. viridis*) growing in the southern areas of the region [11,14].

(**a**) (**b**)

Figure 1. *Fragaria orientalis* Losinsk (oriental strawberry) flowering plants (**a**) and fruit (**b**).

At the same time, in recent decades, agriculturists and breeders have conducted active research on *F. orientalis* domestication, which has led to the creation of some varieties that demonstrated high productivity [15]. In a collection nursery, when transferred from the local flora, *F. orientalis* acclimatizes very quickly (within 2 years), and its development significantly exceeds that of the wild one. In its cultivated forms, the bush of the second year of fruiting is powerful (up to 35 cm or higher), densely leafy, and has a vertical habitus. The beginning of maturation is 1–3 July, which is 10–14 days ahead of wild-growing forms. The average yield per bush is 165 g, and the maximum yield is more than 200 g. The mass of berries in the first collection exceeds those of wild varieties (5–11 g/berry), and the number of ripe berries is incomparably higher and can reach from 150 to 220 per bush, reaching a productivity value of 8–15 t/ha [14,15]. The aroma and taste qualities are close to those of wild berries, but slightly inferior. The shape of the berries varies from cylindrical, ovoid, or heart-shaped to round. The cavity in the berries is absent or insignificant. The pulp is

juicy, whitish in color, tender, and very soft, and the taste is harmonious, with a strong nutmeg aroma. The time of the beginning of berries ripening is medium-early (mid-July), and the type of fruiting is non-repairable (2–3 weeks). Over many years of research, it has been shown that *F. orientalis* is not affected by blotches or powdery mildew [11]. This suggests a profitable possibility for the cultivation of oriental strawberry that will expand the assortment of northern berries.

However, despite the importance of *F. orientalis* fruit as a food product, there is no information regarding the chemical composition, nutritional value, or health benefits of the oriental strawberry. In our opinion, this lack of information does not allow us to reveal the valuable potential of this important Asian crop. Therefore, as part of our ongoing study of wild strawberries [10], the nutritional value, general chemical composition, and sensory characteristics of *F. orientalis* fruits were studied, followed by LC–MS profiling, quantitative evaluation of selected phenolics, and an in vitro antioxidant potential investigation. In addition, various methods of post-harvest storage were applied to evaluate the stability of the selected metabolites and the antioxidant activity of fresh, frozen, and dried fruits of this oriental strawberry.

2. Materials and Methods

2.1. Plant Material and Chemicals

Wild samples of *Fragaria orientalis* ripe fruits were collected in forest populations of Yakutia (Sakha) Republic and Buryatia Republic (Table S1). Cultivated samples of *F. orientalis* were harvested in Buryat Fruit and Plant Nursery located in the vicinity of Ulan-Ude (Russia) (Table S1). Other strawberries were collected in Irkutsk region (*F. viridis*, *F. vesca*) and Buryatia Republic (*F. vesca*) or were harvested in Buryat Fruit and Plant Nursery (*F. ananassa*, cv. Senga Sengana). All species was authenticated by Prof. N.I. Kashchenko (IGEB SB RAS, Ulan-Ude, Russia). The fruits were conditioned in plastic boxes and transported to the laboratory at 4 °C within 2–3 h. The reference compounds were purchased from BioCrick (Chengdu, Sichuan, PRC), ChemFaces (Wuhan, Hubei, PRC), Extrasynthese (Lyon, France), Funakoshi Co. Ltd. (Tokyo, Japan), Sigma-Aldrich (St. Louis, MO, USA), Toronto Research Chemicals (North York, ON, Canada), TransMIT GmbH (Gießen, Germany) or were isolated previously in our laboratory (Table S2). Selected chemicals were from Sigma-Aldrich—acetonitrile for HPLC (cat. No. 34851, ≥99.9%), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), diammonium salt (cat. No. A1888, ≥98%), 2,2-diphenyl-1-picrylhydrazyl radical (cat. No. 281689, ≥97%), formic acid (cat. No. 33015, ≥98%), and trolox (cat. No. 238813, ≥97%). The general workflow included analysis of nutritional value, general chemical composition, sensory evaluation, HPLC-PDA-MS profiling and quantification, antioxidant activity study, and postharvest stability study of chemical composition and total antioxidant potential (Figure 2).

2.2. Analysis of Nutritional Value, General Chemical Composition and Sensory Evaluation

Proximate composition was studied using AOAC assays for analysis of dry matter (AOAC, 930.15), ash (AOAC, 942.05), crude protein (AOAC, 991.02), crude fat (AOAC, 989.05), carbohydrates (AOAC, 935.42), dietary fiber composition (AOAC, 993.19 and 991.42), and titratable acids (AOAC, 942.15) [16]. Free sugars (fructose, glucose, and sucrose) were analyzed by HPLC-DAD procedure in water extraction and purification in Dowex® 50WX8 (H^+-form)/Dowex® 1X8 (Cl^--form) cartridges (Sigma-Aldrich, St. Louis, MO, USA) [17]. The results of proximate composition and free sugars were expressed in g per 100 g of fresh weight of the fruits. Ready-to-use kits for spectrophotometric assays were applied to measure the ascorbic acid (ascorbic acid assay kit, Megazyme, Bray, Ireland; cat. No. K-ASCO), anthocyanins (plant anthocyanin content assay kit, Solarbio, Beijing, China; cat. No. BC1380), catechins (catechins kit, SinaTech, Barcelona, Spain; cat. No. SY4016), proanthocyanidins (proanthocyanidins assay kit—DMAC method, Bioquochem, Asturias, Spain; cat. No. KB03017), flavonoids (plant flavonoids colorimetric assay kit, Elabscience Biotechnology Inc., Houston, TX, USA; cat. No. E-BC-K284-S), and total phenolics (phenolic

compounds colorimetric assay kit, Sigma-Aldrich, St. Louis, MO, USA; cat. No. MAK365). Ellagic acid content was measured by HPLC–UV assay [18] before (free ellagic acid) and after hydrolysis by 1.2 M HCl in 50% methanol medium (total ellagic acid) [19]. The results of ready-to-use kits analysis and ellagic acid determination were expressed in mg per 100 g of fresh weight of the fruits. Sensory evaluation of *Fragaria* fruits were determined according to AHPA guidance on Organoleptic Analysis [20].

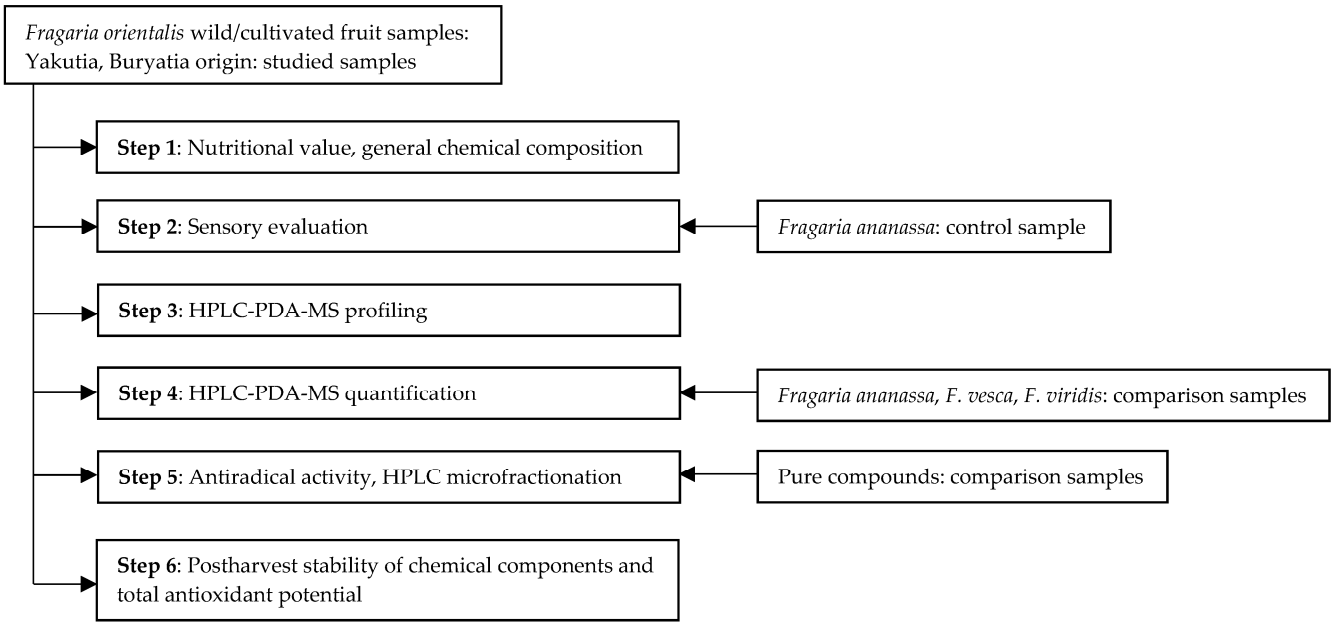

Figure 2. Flow chart for the experimental work with *F. orientalis* fruits.

2.3. Fragaria Fruit Extracts Preparation

To prepare *Fragaria* extracts, the fresh fruits was homogenized in a Grindomix GM 200 grinder (Retsch GmbH, Haan, Germany), and the pulp (100 g) was sonicated twice with methanol (250 mL) at 40 °C by Sapphire 2.8 bath (Sapphire Ltd., Moscow, Russia) for 30 min, ultrasound power 100 W, and frequency 35 kHz [10]. The methanolic extracts were filtered through cellulose, concentrated in vacuo until dryness, and stored at 4 °C before use for HPLC analysis and antioxidant activity study. Before analysis, dry extract (100 mg) was dissolved in 10 mL 50% methanol using measuring flask (10 mL) and filtered through 0.22 µm syringe filters.

2.4. High-Performance Liquid Chromatography with Photodiode Array Detection and Electrospray Ionization Triple Quadrupole Mass Spectrometric Detection (HPLC-PDA-ESI-tQ-MS) Metabolite Profiling

The previously described method used high-performance liquid chromatography with photodiode array detection, and electrospray ionization triple quadrupole mass spectrometric detection (HPLC-PDA-ESI-tQ-MS) was applied for the metabolite profiling of *F. orientalis* extracts [10]. The method was equipped by a liquid chromatograph, LC-20 Prominence, coupled with a photodiode array detector, SPD-M30A (wavelength range of 200–600 nm), triple-quadrupole mass spectrometer, LCMS 8050 (all Shimadzu, Columbia, MD, USA), and GLC Mastro column (2.1 × 150 mm, 3 µm; Shimadzu, Kyoto, Japan). The negative electrospray ionization was used for the mass spectrometric detection of gallic acid derivatives, hydroxycinnamates, catechins, proanthocyanidins, ellagic acid derivatives, ellagitannins, flavonoids, and triterpenes, and the positive electrospray ionization was used for anthocyanins. The LC-MS system was managed by LabSolution's workstation software equipped with the inner LC-MS library. The integrated analysis of retention time, ultraviolet, and mass spectra data, after comparison with the reference standards and literature data, was used for the identification of metabolites.

2.5. HPLC-ESI-tQ-MS Metabolite Quantification

To quantify 20 phenolic compounds and ascorbic acid, the HPLC-PDA-ESI-tQ-MS conditions were used (Section 2.4) (Table S3). In total, 17 reference standards were separately weighed (10 mg) and dissolved in the methanol–DMSO mixture (1:1) in volumetric flasks (10 mL), preparing the stock solution (1000 μg/mL) used for the calibration curve building. The calibration solution (1–100 μg/mL) chromatographed in known HPLC-PDA-ESI-tQ-MS conditions and mass spectral data was used to create 'concentration–mass spectrometric peak area' correlation. The principal validation criteria, including correlation coefficients (r^2), standard deviation (S_{YX}), limits of detection (LOD), limits of quantification (LOQ), and linear ranges, were found using the known method [21] (Table S3). Five HPLC runs were sufficient for the quantitative analyses, and the results were expressed as mean values ± standard deviation (S.D.).

2.6. Principal Component Analysis (PCA)

PCA, based on a data matrix (20 quantitative markers × 45 samples), was performed using Graphs 2.0 utility for Microsoft Excel (Komi NTc URO RAN, Syktyvkar, Russia) to generate an overview for groups clustering.

2.7. Antioxidant Potential

Ready-to-use kits of in vitro spectrophotometric assays of antiradical activity were used to measure scavenging potential of *Fragaria* extracts against 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH$^{\bullet}$; DPPH antioxidant assay kit, BioVision Inc., Waltham, MA, USA; cat. No. K2078) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) cation radicals (ABTS$^{+\bullet}$; ABTS assay kit, Bioquochem, Asturias, Spain; cat. No. KF01002). All assays used trolox as a reference standard (methanolic solution 0.5–100 μg/mL), and the calibration curve was created by plotting the trolox concentration (μg/mL) vs. the absorbance. The values of antioxidant parameters were expressed as μmol trolox-equivalents/g of dry weight for *F. orientalis* extracts and mmol trolox-equivalents/g of dry weight or μg/mL (half maximal inhibitory concentration, IC_{50}) for the pure compounds. All the analyses were carried out five times, and the data were expressed as mean value ± standard deviation (SD).

2.8. HPLC Microfractionation with Post-Column DPPH$^{\bullet}$ Radicals Scavenging

Described HPLC-PDA-ESI-tQ-MS conditions (Section 2.4) were applied to separate the increased volume of *F. orientalis* fruit extract (50 μL; 40 mg/mL). The eluates (50 μL) were collected every 25 s by Shimadzu FRC-10A automated fraction collector and dried under a stream of N_2. The eluate residue was dissolved in 50 μL of 60% methanol, mixed with 0.02% methanol solution of DPPH$^{\bullet}$ radicals, incubated for 10 min (25 °C), and the absorbance was measured at 520 nm. The most active DPPH$^{\bullet}$ radicals scavenging eluates resulted in most decoloration, instead of the inactive eluates, giving a strong coloration. The value of 0% antioxidant activity (or 0% DPPH$^{\bullet}$ radicals scavenging) was measured for the eluate, with a retention time of 0.50–0.75 min.

2.9. F. orientalis Fruit Storage Experiment

2.9.1. Storage of Fresh *F. orientalis* Fruits at Cold and Room Temperatures

Seven and three portions of the fresh cultivated *F. orientalis* fruits (ca. 200 g; sample BC-1) were deposited into the individual polystyrene bags (400 mL) and incubated at 4 °C (7 days) or 20 °C (3 days), respectively, in a ventilated MK 53 thermostat (BINDER GmbH, Tuttlingen, Germany). Twenty-grams portions (5 replications) of stored fruits were taken out for analysis every 24 h, with standard extraction/analysis procedure applied—extraction as described in Section 2.3, and analysis of quantitative content of phenolic compounds and ascorbic acid (Sections 2.4 and 2.5) or determination of the total antioxidant potential by coulometric assay (Section 2.10).

2.9.2. Storage of Frozen *F. orientalis* Fruits and Thawing

Eight portions of the fresh cultivated *F. orientalis* fruits (500 g; sample BC-1) were deposited into individual polystyrene bags (1000 mL), frozen at −20 °C for 3 days at shock-freeze chamber BCF 20.1.T1 (Polair, Moscow, Russia), after which, the 4-year experiment was begun. Four of them were used for analysis without thawing (B-samples) and four were analyzed after thawing (A-samples). Twenty-gram portions (5 replications) of B-samples were taken out for analysis every 1 year and milled in cryogenic homogenizer GL-P-300/10000 (Petrolazer, St. Petersburg, Russia), with standard extraction/analysis procedure applied. Twenty-gram portions (5 replications) of B-samples were taken out for analysis every 1 year and thawed at 20 °C for 30 min, with standard extraction/analysis procedure applied.

2.9.3. Storage of Freeze-dried *F. orientalis* Fruits at Cold and Room Temperatures

To dry *F. orientalis* fruits, 1 kg of fresh cultivated *F. orientalis* fruits (sample BC-1) were subjected to (1) sun drying in the drying tray into the open sun for 20 days; (2) heat drying at the drying tray in the ventilated heat oven (40 °C, 5–7 days); (3) microwave drying in the microwave oven SmartSensor Microwave ME6144ST (Samsung, Seoul, South Korea; 1000 W, 5 min); and (4) freeze drying by the freeze-dryer BK-FD10PT (Biobase Biodusty Co., Jinan, China) until the humidity content 4–5%.

Four portions of the freeze-dried *F. orientalis* fruits (ca. 10 g) were deposited into the individual polystyrene bags (100 mL) and incubated at 4 °C or 20 °C in a ventilated MK 53 thermostat (BINDER GmbH, Tuttlingen, Germany) for 4 years. One-gram portions (5 replications) of stored fruits were taken out for analysis every year, extracted three times with 50% methanol (30 L) and sonification for 15 min at 40 °C (100 W, 35 kHz), and filtrated through a cellulose filter in volumetric flasks (100 mL); the final volume was reached 100 mL, with 50% methanol, and filtered through a 0.22-µm PTFE syringe filter before LC-MS analysis (Sections 2.4 and 2.5) or determination of the total antioxidant potential by coulometric assay (Section 2.10).

2.10. Total Antioxidant Potential

Previously described coulometric assay was used to determine the total antioxidant potential, based on a modified bromine radical scavenging assay with electrogenerated bromine radicals by potentiostat Expert-006 (Econics Expert Ltd., Moscow, Russia), four-electrode two-compartment electrochemical cell, bare platinum foil (working electrode), and a platinum wire (auxiliary electrode) [10]. Values are expressed as means obtained from ten independent experiments.

2.11. Statistical Analysis

Statistical analyses were performed by one-way analysis of variance, and the significance of the mean difference was determined by Duncan's multiple range test. Differences at $p < 0.05$ were considered statistically significant. The results are presented as the mean ± S.D. The linear regression analysis and generation of calibration graphs were conducted using Advanced Grapher 2.2 (Alentum Software, Inc., Ramat-Gan, Israel).

3. Results and Discussion

3.1. Nutritional Value, General Chemical Composition, and Sensory Evaluation of Fragaria orientalis Fruits

Strawberry fruits, as a food product, require the mandatory definition of nutritional value that is known for *F. ananassa* [5,22–31] and *F. vesca* [32–34]. The nutritional compositions of wild *F. orientalis* fruits collected in two Siberian localities (Yakutia and Buryatia) and cultivated samples farmed in Buryatia locality were more or less similar and demonstrated a high content of dry matter, which was 16.0–23.1 g/100 g for wild and 17.8–20.7 g/100 g for cultivated samples (Tables 1 and S4). The major fruit components were carbohydrates (9.0–14.0 g/100 g), followed by lipids (0.8–1.4 g/100 g), ash (0.6–1.4 g/100 g), and

proteins (0.4–0.6 g/100 g). Fruits of *F. ananassa* have a smaller content of dry matter (8–14 g/100 g) [22,23,25], carbohydrates (7–12 g/100 g) [22,31], lipids (0.1–0.3 g/100 g) [22], ash (0.4–0.7 g/100 g) [22,26], and proteins (0.5–0.8 g/100 g) [22], while *F. vesca* fruits were most similar to *F. orientalis* [32–34]. The basic simple carbohydrates of *F. orientalis* fruits were fructose (1.5–2.3 g/100 g), glucose (1.4–2.0 g/100 g), and sucrose (1.4–2.1 g/100 g). The level of total dietary fibers varied from 4.6 to 6.1 g/100 g in wild samples and from 5.4 to 5.9 g/100 g in cultivated samples, with soluble and insoluble dietary fibers accounting for 4.1–5.2 g/100 g and 0.5–1.1 g/100 g, respectively. Analysis of 24 American cultivars of *F. ananassa* showed higher fructose (2.0–3.7 g/100 g) and glucose levels (1.5–2.9 g/100 g) and a lower sucrose content (0.1–1.1 g/100 g) [30]. Italian cultivars of *F. vesca* were more sugary, with levels of fructose, glucose, and sucrose in fruits of 4.2–5.7, 3.8–5.2, and 3.1–3.7 g/100 g, respectively [33]; however, the wild Portugal samples of *F. vesca* showed close carbohydrate levels [34].

Table 1. Nutritional and chemical composition of fresh wild and cultivated *F. orientalis* fruits (± S.D.).

Parameter	Wild Samples, Yakutia	Wild Samples, Buryatia	Cultivated Samples, Buryatia
Dry matter, g/100 g	17.17 ± 1.15 ^{a}	20.50 ± 2.25 ^{a}	19.70 ± 1.45 ^{a}
Ash, g/100 g	0.80 ± 0.15 ^{a}	1.04 ± 0.25 ^{a}	0.78 ± 0.10 ^{a}
Protein, g/100 g	0.44 ± 0.05 ^{a}	0.53 ± 0.10 ^{a}	0.48 ± 0.10 ^{a}
Lipids, g/100 g	0.99 ± 0.15 ^{a}	1.19 ± 0.20 ^{a}	0.93 ± 0.15 ^{a}
Carbohydrates, g/100 g	9.73 ± 0.70 ^{a}	12.43 ± 1.50 ^{b}	10.70 ± 0.90 ^{ab}
Fructose, g/100 g	1.84 ± 0.35 ^{a}	2.04 ± 0.25 ^{a}	1.94 ± 0.10 ^{a}
Glucose, g/100 g	1.73 ± 0.30 ^{a}	1.87 ± 0.15 ^{a}	1.75 ± 0.10 ^{a}
Sucrose, g/100 g	1.75 ± 0.30 ^{a}	1.94 ± 0.15 ^{a}	1.77 ± 0.20 ^{a}
Total dietary fibers, g/100 g	5.03 ± 0.35 ^{a}	5.79 ± 0.40 ^{a}	5.64 ± 0.25 ^{a}
Soluble dietary fibers, g/100 g	4.31 ± 0.20 ^{a}	4.91 ± 0.40 ^{a}	4.97 ± 0.25 ^{a}
Insoluble dietary fibers, g/100 g	0.72 ± 0.15 ^{a}	0.88 ± 0.25 ^{a}	0.67 ± 0.10 ^{a}
Titratable acids, g/100 g	1.02 ± 0.30 ^{a}	0.95 ± 0.15 ^{a}	0.83 ± 0.05 ^{a}
Ascorbic acid, mg/100 g	120.59 ± 32.6 ^{a}	143.42 ± 23.4 ^{a}	122.12 ± 12.3 ^{a}
Ellagic acid free, mg/100 g	12.12 ± 3.35 ^{ab}	20.49 ± 7.90 ^{b}	11.38 ± 1.95 ^{a}
Ellagic acid total, mg/100 g	234.98 ± 42.05 ^{a}	362.28 ± 41.9 ^{a}	321.23 ± 17.00 ^{a}
Anthocyanins, mg/100 g	10.48 ± 3.85 ^{a}	10.65 ± 3.00 ^{a}	14.63 ± 3.20 ^{a}
Catechins, mg/100 g	3.91 ± 1.20 ^{a}	3.94 ± 0.55 ^{a}	4.12 ± 0.40 ^{a}
Proanthocyanidins, mg/100 g	6.33 ± 1.30 ^{a}	5.29 ± 1.10 ^{a}	6.00 ± 0.55 ^{a}
Flavonoids, mg/100 g	4.50 ± 1.95 ^{a}	3.57 ± 0.60 ^{a}	3.98 ± 0.35 ^{a}
Total phenolics, mg/100 g	918.33 ± 205.91 ^{a}	1054.33 ± 304.60 ^{a}	822.43 ± 89.3 ^{a}

Values with different letters (a, b) indicate statistically significant differences among groups of same parameters at $p < 0.05$ by one-way ANOVA.

The titratable acid content in *F. orientalis* fruit was 0.8–1.4 mg/100 g, which resembled those in the European, Asian, and American cultivars of *F. ananassa* (0.5–1.4 mg/100 g) [22,23,25,26,29,30] and was much lower than that in the wild *F. vesca*, containing 7.5 mg/100 g of organic acids [34]. Ascorbic acid contents in wild samples of *F. orientalis* from Yakutia and Buryatia were 89.8–167.1 mg/100 g, with average levels of 120.59 and 143.42 mg/100 g for the fresh fruits, respectively, and the cultivated fruits showed a similar content (109.6–134.2 mg/100 g). This amount is considered high for strawberries, as evidenced by the known data for *F. ananassa* (25–112 mg/100 g) [23,26,27,29,30] and *F. vesca* (28–45 mg/100 g) [32–34].

Phenolic compounds of strawberries are the main bioactive phytochemicals of *Fragaria* responsible for the antioxidant potential of the fruit [35], and the basic phenolics of the genus are ellagic acids, ellagitannins, anthocyanins, flavonoids, catechins, and procyanidins [2]. Ellagic acid, as a parent compound of ellagitannins, is an obligatory component of strawberry phenolics, and its content is 0.4–4.1 mg/100 g in the free state and up to 261 mg/100 g in the bound state [36]. The free ellagic acid content in *F. orientalis* fruit was 8.6–28.7 mg/100 g, and the total ellagic acid content reached 281–406 mg/100 g in wild samples and 340 mg/100 g in cultivated samples, which is a very high level for known wild

and cultivated strawberries. Anthocyanins, as primary pigments of strawberries, varied in wild *F. orientalis* fruits from 7.3 to 15.0 mg/100 g and from 10.8 to 17.2 mg /100 g in cultivated samples. The known anthocyanin content in *F. ananassa* was lower in Portugal cultivars (2.1–2.4 mg/100 g) [22] and Pakistan cultivars (1–3.5 mg/100 g) [26], as well as a similar level in Romanian cultivars (19–24 mg/100 g) [23] and at a higher amount in Italian cultivars (310–610 mg/100 g) [24]. The wild and cultivated fruits of *F. vesca* and *F. viridis* have anthocyanin contents of 2–10 mg/100 g [37,38] and 3–14 mg/100 g [10], respectively, which indicates that small strawberries are less pigmented than the large-fruited strawberry hybrids. The contents of catechins, procyanidins, and flavonoids in wild and cultivated *F. orientalis* fruits were similar and amounted to 2.6–5.0, 4.5–7.8, and 2.2–6.1 mg/100 g, respectively. The total content of catechins and procyanidins in *F. vesca* cv. Mara de Bois was 1–4 mg/100 g, and the flavonoid content was 4–6 mg/100 g [39]. The catechin and procyanidin content in Norway cultivars of *F. ananassa* was 2–8 and 4–16 mg/100 g, respectively, while the amount of quercetin derivatives reached 0.5–3.4 mg/100 g [40]. In fruits of *F. viridis*, the amount of catechins and procyanidins was 9–29 mg/100 g in various stages of ripeness, while that of the flavonoids was higher, i.e., 104–124 mg/100 g [10].

The total phenolic contents in wild and cultivated *F. orientalis* fruits were 714–1463 mg/100 g and 714–893 mg/100 g, respectively, which were higher than those in the *F. ananassa* cultivars harvested in Romania (208–250 mg/100 g) [23] and Italy (128–232 mg/100 g) [27]. Similar phenolic contents were found in Indian (380–888 mg/100 g) [29] and Portuguese (607–1314 mg/100 g) [22] samples of *F. ananassa* and in Italian cultivars of *F. vesca* (717–727 mg/100 g) [33].

Previous studies found that *F. orientalis* fruit had similar nutritional composition to cultivated strawberries (e.g., *F. ananassa* and *F. vesca*) and was a source of dietary fibers, ascorbic acid, and phenolic compounds. The sensory evaluation of *F. orientalis* fruits showed high values for color, flavor, juiciness, and overall preference for wild and cultivated samples, with a slight advantage for cultivated fruits (Figure 3, Tables S5 and S6), which indicates the prospects for their use as dietary products, along with other strawberries.

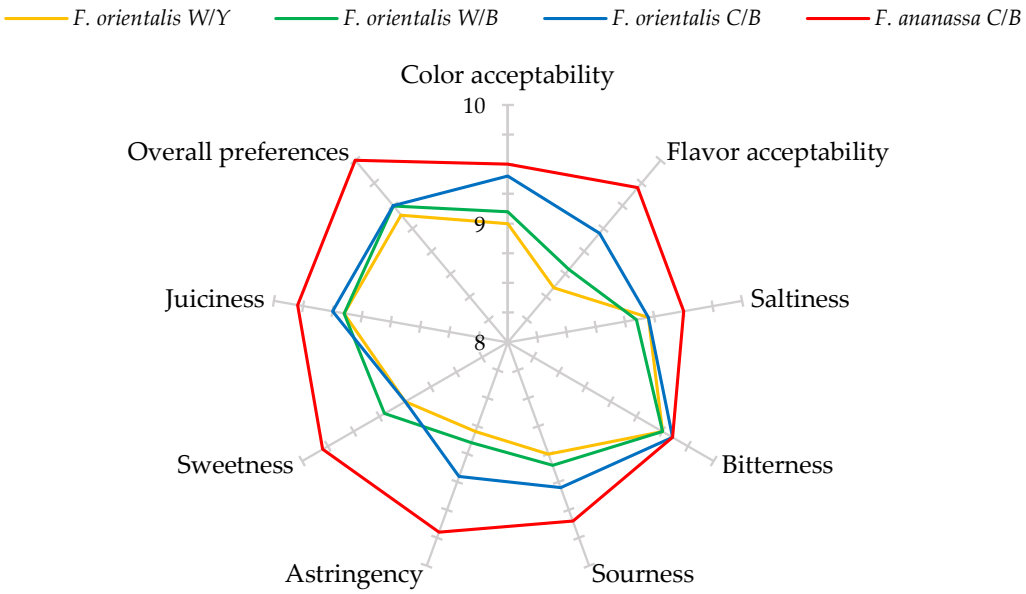

Figure 3. Sensory profiles of *F. orientalis* fruits, according to estimates by 60 participants. The preference scale ranged from 0 (greatest imaginary dislike) to 10 (greatest imaginary like). Fruits of *F. ananassa* were used as a reference sample. Samples notation: B—Buryatia origin; C—cultivated; W—wild; Y—Yakutia origin.

3.2. HPLC-PDA-tQ-ESI-MS Profile of *F. orientalis* Fruits

The application of high-performance liquid chromatography with photodiode array and electrospray ionization triple quadrupole mass spectrometric detection (HPLC-PDA-ESI-tQ-ESI-MS) resulted in the characterization of 65 compounds in the extract of the *F. orientalis* fruit (Figure 4, Table S7). Forty-five compounds were identified after comparison of the retention times and ultraviolet and mass spectra with reference standards or literature data [41–48], and twenty metabolites were tentatively identified. All described compounds were found in *F. orientalis* for the first time.

The most hydrophilic compounds have the lowest retention times and are eluted as two peaks of hexosyl-hexose (*m*/*z* 341; **1**) and hexose (*m*/*z* 179; **2**) and five organic acids, including citric acid (*m*/*z* 191; **3**), malic acid (*m*/*z* 133; **4**), tartaric acid (*m*/*z* 149; **5**), fumaric acid (*m*/*z* 115; **6**), and ascorbic acid (*m*/*z* 175; **7**). All compounds are the usual components of the strawberry metabolome [2].

Gallic acid (**10**) gave a specific UV absorbance and MS pattern (*m*/*z* 169), and it had two glycosides in *F. orientalis* fruit, i.e., 1-*O*-galloyl glucose (**8**) and isomeric galloyl hexose **9**. The parent compound **10** and monogalloylated glucose have been found in *F. ananassa* [49] and *F. viridis* [10].

Two hydroxycinnamates, 4-*O*-caffeoylquinic acid (**12**) and 5-*O*-caffeoylquinic acid (**16**), were detected in the *F. orientalis* extract. Both acids are components of *F. viridis* [10], and the acid **16** was previously found in *F. ananassa* and *F. vesca* [50].

Monomeric flavan-3-ols or catechins of the *F. orientalis* fruit (i.e., catechin (**19**) and epicatechin (**20**)) showed a deprotonized fragment at *m*/*z* 289. The reference standards allowed us to identify two catechin dimers, procyanidin B2 (**14**) and B4 (**17**) (*m*/*z* 577), and one trimer, procyanidin C2 (**24**; *m*/*z* 865). Compound **22** was isomeric to **24** and characterized as the catechin/epicatechin trimer. Monomeric and oligomeric flavan-3-ols are typical for *Fragaria* organs, including the fruits [2].

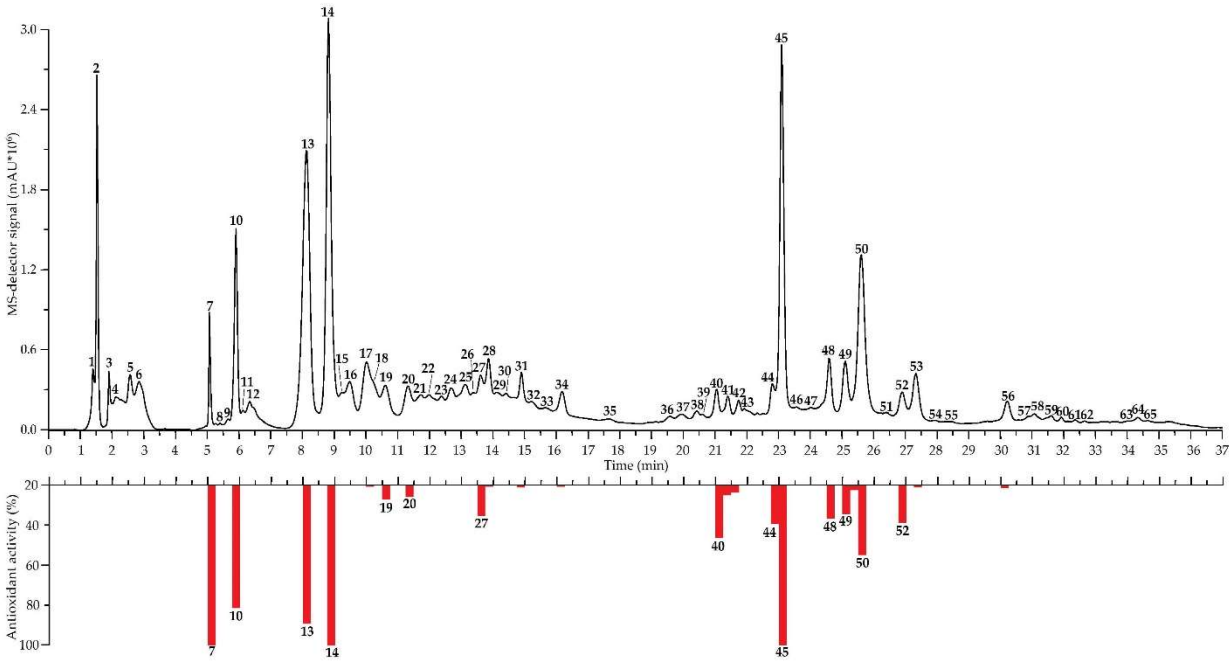

Figure 4. High-performance liquid chromatography with electrospray ionization triple quadrupole mass spectrometric detection (HPLC-ESI-tQ-ESI-MS) chromatogram (base peak intensity chromatogram, negative ionization) of an extract of *F. orientalis* fruit (on top; compounds are numbered as listed in Table 2) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) scavenging activity of the HPLC fraction of *F. orientalis* fruit extract. Red bars show the antiradical activity (as percentage) in the probe after reaction with the DPPH$^{\bullet}$ radical solution.

Table 2. Quantitative content of 20 phenolic compounds in the fruits of wild (W) and cultivated (C) *Fragaria* species, mg/100 g FW (± S.D.).

Compound	*F. orientalis* W (*n* = 100)	*F. orientalis* C (*n* = 40)	*F. viridis* W (*n* = 30)	*F. vesca* W (*n* = 40)	*F. ananassa* C (*n* = 15)
Gallic acid	64 ± 24 ^{c}	42 ± 14 ^{c}	2 ± 1 ^{a}	13 ± 6 ^{b}	3 ± 1 ^{a}
Ellagitannins					
Strictinin	130 ± 30 ^{d}	73 ± 12 ^{c}	12 ± 2 ^{b}	5 ± 2 ^{a}	0
Lambertianin C	13 ± 7 ^{b}	10 ± 2 ^{b}	102 ± 20 ^{d}	30 ± 8 ^{c}	5 ± 2 ^{a}
Agrimoniin	161 ± 29 ^{c}	105 ± 14 ^{b}	139 ± 8 ^{c}	137 ± 26 ^{c}	18 ± 6 ^{a}
1-*O*-Ellagoyl gentiobiose	4 ± 2 ^{b}	2 ± 1 ^{ab}	0	1 ± 0 ^{a}	0
1-*O*-Ellagoyl glucose	2 ± 1	0	0	0	0
Ellagic acid	19 ± 8 ^{b}	13 ± 5 ^{b}	10 ± 2 ^{b}	13 ± 6 ^{b}	2 ± 1 ^{a}
Subtotal	330 ± 59 ^{c}	203 ± 21 ^{b}	262 ± 18 ^{c}	185 ± 37 ^{b}	24 ± 12 ^{a}
Catechins and proanthocyanidins					
Procyanidin B2	5 ± 2 ^{b}	4 ± 2 ^{b}	2 ± 1 ^{ab}	4 ± 2 ^{b}	1 ± 0 ^{a}
Catechin	2 ± 1 ^{ab}	1 ± 0 ^{a}	4 ± 2 ^{b}	2 ± 1 ^{ab}	1 ± 0 ^{a}
Epicatechin	2 ± 1 ^{a}	1 ± 0 ^{a}	0	1 ± 0 ^{a}	1 ± 0 ^{a}
Subtotal	9 ± 4 ^{b}	6 ± 2 ^{a}	6 ± 2 ^{a}	7 ± 3 ^{ab}	3 ± 1 ^{a}
Anthocyanidins					
Cyanidin 3-*O*-glucoside	2 ± 1 ^{a}	2 ± 1 ^{a}	2 ± 1 ^{a}	1 ± 0 ^{a}	3 ± 1 ^{a}
Pelargonidin 3-*O*-glucoside	9 ± 4 ^{b}	11 ± 2 ^{b}	2 ± 1 ^{a}	1 ± 0 ^{a}	40 ± 10 ^{c}
Pelargonidin *O*-malonyl-hexoside	3 ± 1 ^{b}	4 ± 2 ^{b}	0	0	1 ± 0 ^{a}
Pelargonidin di-*O*-malonyl-hexoside	5 ± 2 ^{a}	4 ± 2 ^{a}	0	0	3 ± 1 ^{a}
Subtotal	18 ± 5 ^{b}	21 ± 4 ^{b}	4 ± 2 ^{a}	2 ± 1 ^{a}	50 ± 11 ^{c}
Flavonoids					
Kaempferol 3-*O*-rutinoside	2 ± 1 ^{a}	2 ± 1 ^{a}	6 ± 2 ^{b}	1 ± 0 ^{a}	2 ± 0 ^{a}
Kaempferol 3-*O*-glucoside	2 ± 1 ^{a}	1 ± 0 ^{a}	7 ± 4 ^{b}	2 ± 1 ^{a}	2 ± 1 ^{a}
Quercetin 3-*O*-arabinoside	2 ± 1 ^{a}	2 ± 1 ^{a}	1 ± 0 ^{a}	1 ± 0 ^{a}	0
Quercetin 3-*O*-(6″-*O*-acetyl)-glucoside	2 ± 1 ^{ab}	1 ± 0 ^{a}	3 ± 1 ^{b}	1 ± 0 ^{a}	1 ± 0 ^{a}
Kaempferol 3-*O*-(6″-*O*-*p*-coumaroyl)-glucoside	3 ± 1 ^{ab}	4 ± 1 ^{b}	2 ± 1 ^{a}	3 ± 1 ^{ab}	1 ± 0 ^{a}
Quercetin *O*-acetyl-*O*-malonyl-*O*-hexoside	2 ± 1 ^{a}	1 ± 0 ^{a}	0	0	0
Subtotal	11 ± 4 ^{ab}	9 ± 2 ^{a}	19 ± 4 ^{b}	8 ± 2 ^{a}	6 ± 2 ^{a}
Total phenolic compounds	431 ± 78 ^{c}	281 ± 37 ^{b}	292 ± 21 ^{b}	215 ± 40 ^{b}	86 ± 22 ^{a}

Values with different letters (a–d) indicate statistically significant differences among groups of same compound content at $p < 0.05$ by one-way ANOVA.

Ellagic acid (**50**) was found in *F. orientalis* fruits in free and glycosidic form, including ellagoyl glucose (**49**; *m*/*z* 463), ellagoyl gentiobiose (**48**; *m*/*z* 625), and two desoxyhexosides, **57** and **58**. Compound **57** gave a deprotonated ion with *m*/*z* 461, which further lost desoxyhexosyl fragments (146 amu) and one methyl fragment (14 amu), which is typical for the ellagic acid *O*-methyl ester-*O*-desoxyhexoside [10,46]. Derivative **58** had a similar cleavage path and gave ions with *m*/*z* 475, 329, and 301, which indicated its possible structure as the ellagic acid *O*-methyl ester-*O*-desoxyhexoside [40]. Compounds with similar spectral patterns were found in *F. viridis* [10]. In addition to glycosides of ellagic acid, 12 ellagitannins were detected in the *F. orientalis* fruit, including pedunculagin (**11**), strictinin (**13**), castalagin (**23**), sanguiin H2 (**26**), sanguiin H6 (**38**), sanguiin H10 (**39**), lambertianin C (**40**), and agrimoniin (**45**), which were identified after comparison of spectral characteristics with reference standards. Isomers of pedunculagin (**15**, **21**; *m*/*z* 783), strictinin (**18**; *m*/*z* 633), and castalagin (**25**; *m*/*z* 933) showed similar spectral patterns, but were at other retention times. Compound **46** with *m*/*z* 1018 $[M\text{-}2H]^{2-}$ gave a series of ions in the MS^2 spectrum at *m*/*z* 1691, 1567, 1265, 1209, 935, 783, 633, 481, and 301, which are typical for fragariin A, an ellagitannin found in *F. ananassa* [45] and *F. viridis* [10].

Eight anthocyanins found in *F. orientalis* fruits were glycosides of cyanidin (λ_{max} 515 nm; aglycone fragment *m*/*z* 285; compounds **27**, **30**, **33**), pelargonidin (λ_{max} 503 nm; aglycone fragment *m*/*z* 269; compounds **28**, **31**, **34**), and peonidin (λ_{max} 520 nm; aglycone fragment *m*/*z* 299; compounds **29**, **32**). Cyanidin-3-*O*-glycoside (**27**), pelargonidin-3-*O*-glycoside (**28**), peonidin-3-*O*-glycoside (**29**), and cyanidin-3-*O*-(6′′-O-malonyl)-glycoside (**30**) were identified using reference standards. The mass spectra of **31** and **32** demonstrated the sequential cleavage of malonyl (86 amu) and hexose (162 amu) fragments, revealing aglycone fragments at *m*/*z* 285 and 299, respectively, indicating the most likely structures as pelargonidin *O*-malonyl-hexoside (**31**) and peonidin *O*-malonyl-hexoside (**32**). Compounds **33** and **34** were compared, relative to compounds **30** and **31**, respectively, which showed

the presence of additional malonyl fragments; this result suggested that compounds **33** and **34** were cyanidin di-*O*-malonyl-hexoside (**33**; *m*/*z* 619) and pelargonidin di-*O*-malonyl-hexoside (**34**; *m*/*z* 603), respectively. The anthocyanins with identified, and tentative structures were previously found in various *Fragaria* species, such as *F. ananassa*, *F. vesca*, and *F. viridis* [2].

Flavonoids of *F. orientalis* fruits were non-acylated and acylated flavonols and derivatives of quercetin and kaempferol. Identified non-acylated quercetins were glycosides, which gave the aglycone ion at *m*/*z* 301, with various carbohydrate moieties, such as quercetin 3-*O*-sophoroside (**35**; *m*/*z* 625→463, 301), quercetin 3-*O*-glucoside (**36**; *m*/*z* 463→301), quercetin 3-*O*-glucuronide (**37**; *m*/*z* 477→301), and quercetin 3-*O*-arabinoside (**44**; *m*/*z* 433→301). Acylated quercetin glycosides included quercetin 3-*O*-(6″-*O*-*p*-coumaroyl)-glucoside (**51**), which showed a specific hypsochromic shift of band I in the UV spectrum and the loss of *p*-coumaroyl fragments in the mass spectrum (*m*/*z* 609→463), quercetin 3-*O*-(6″-*O*-acetyl)-glucoside (**52**), and quercetin 3-*O*-(2″-*O*-acetyl)-glucoside (**54**), without changes in the UV spectrum, but with a specific loss of acetyl (42 amu; *m*/*z* 505→463) and quercetin 3-*O*-(2″,6″-di-*O*-acetyl)-glucoside (**63**), which is comparable to **52** and **54**, but has an extra loss of acetyl (*m*/*z* 547→505, 463). Compound **56**, with a deprotonated ion at *m*/*z* 591, demonstrated loss of malonyl and acetyl fragments (*m*/*z* 591→549, 505, 463) typical for the quercetin *O*-acetyl-*O*-malonyl-*O*-hexoside found in *F. viridis* [10]. Six kaempferol glycosides were identified as kaempferol 3-*O*-rutinoside (**41**; *m*/*z* 593→447, 285), kaempferol 3-*O*-glucoside (**42**; *m*/*z* 447→285), kaempferol 3-*O*-glucuronide (**43**; *m*/*z* 461→285), kaempferol 3-*O*-rhamnoside (**47**; *m*/*z* 431→285), kaempferol 3-*O*-(6″-*O*-*p*-coumaroyl)-glucoside (**53**; *m*/*z* 593→447, 285), and kaempferol 3-*O*-(6″-*O*-acetyl)-glucoside (**55**; *m*/*z* 489→447, 285). Derivatives of quercetin and kaempferol are typical for Rosaceous plants and have been identified in the *Fragaria* genus many times [2].

Compounds **64** and **65**, after comparison with the reference standards, were identified as triterpenes tormentic (*m*/*z* 487) and pomolic acids (*m*/*z* 471), respectively. Both acids have glycosidic derivatives with one and two hexose fragments, as indicated by the mass spectral patterns of compounds **59** (*m*/*z* 811→649, 487; tormentic acid di-*O*-hexoside), **60** (*m*/*z* 795→633, 471; pomolic acid di-*O*-hexoside), **61** (*m*/*z* 649→487; tormentic acid *O*-hexoside), and **62** (*m*/*z* 633→471; pomolic acid *O*-hexoside). Tormentic acid has been found in the fruits of *F. ananassa* [51], and the glycosides of tormentic and pomolic acids have been found in *F. viridis* [10].

Thus, using HPLC-PDA-ESI-tQ-ESI-MS analysis, we found sixty-five metabolites, including carbohydrates, organic acids, gallic acid derivatives, hydroxycinnamates, catechins, proanthocyanidins, ellagic acid derivatives, ellagitannins, anthocyanins, flavonoids, and triterpenes. Many compounds have been previously found in *F. ananassa*, *F. vesca*, and *F. viridis*, indicating the metabolic similarity of *F. orientalis* with commonly known strawberries.

3.3. Quantification of Selected Phenolics in F. orientalis Fruits

To quantify the principal compounds, we used HPLC-ESI-tQ-ESI-MS data, which allowed us to determine the content of 20 phenolic compounds in *F. orientalis* fruit extracts. Gallic acid, six ellagitannins, procyanidin B2, two catechins, four anthocyanidins, and six flavonoids were chosen as quantitative markers for the samples of wild and cultivated *F. orientalis* fruits. In addition, the compositions of two wild species, *F. viridis* and *F. vesca*, and the cultivated *F. ananassa* were studied. Quantitative data on 20 phenolic compounds in *F. orientalis* fruits showed the similarities of the wild and cultivated samples; however, the total phenolic compound content in the wild fruits (368–524 mg/100 g; average of 431 mg/100 g) was higher than that in the cultivated fruits (240–315 mg/100 g; average of 281 mg/100 g) by approximately 1.5 times (Tables 2 and S8). The mean level of the main phenolic group, ellagitannins, was 330/203 mg/100 g (wild/cultivated), followed by gallic acid (64/42 mg/100 g), anthocyanidins (18/21 mg/100 g), flavonoids (11/9 mg/100 g), and catechins and proanthocyanidins (9/6 mg/100 g). The predominant ellagitannins were

strictinin and agrimoniin, with variation ranges of 108–168 and 134–193 mg/100 g in the wild samples and 60–84 and 92–120 mg/100 g in the cultivated samples, respectively.

Previous studies showed that a higher content of phenolic compounds and vitamins was found in apricot (*Prunus armeniaca*) fruits grown under wild conditions, compared to cultivated fruits [52]. The same results were also obtained for wild cape gooseberry (*Physalis peruviana*) [53] and blackberry (*Rubus fruticosus*) [54].

Comparative analysis of phenolic compounds in other strawberry fruits growing in wild conditions or cultivated in Siberia suggested a lower level of identified phenolics in wild *F. vesca* (175–256 mg/100 g; average of 215 mg/100 g) and *F. viridis* (270–312 mg/100 g; average of 292 mg/100 g), as well as in cultivated *F. ananassa* (62–107 mg/100 g; average of 86 mg/100 g). Ellagitannins were basic compounds in *F. viridis* (243–280 mg/100 g) and *F. vesca* (145–219 mg/100 g), although the level was lower than in *F. orientalis*. In fruits of *F. ananassa*, we predominantly found anthocyanidins, 39–61 mg/100 g, which was the highest value in all studied strawberries. Flavonoid content in *F. viridis* varied from 14 to 23 mg/100 g, which was higher than that in *F. orientalis* (5–15 mg/100 g), *F. vesca* (6–10 mg/100 g), and *F. ananassa* (4–8 mg/100 g). However, despite the slight differences, *F. orientalis* fruits have the highest total level of phenolic compounds among all strawberries grown or cultivated in Siberia.

3.4. Antioxidant Potential of F. orientalis Fruits and Selected Compounds

Owing to their ability to accumulate various phenolics and ascorbic acid, strawberry fruits are a proven source of antioxidants [2]. According to our research, *F. orientalis* is a promising antioxidant food, owing to its high levels of gallic acid, ellagitannins, and ascorbic acid. This assumption was confirmed by a series of in vitro studies, which found that the *F. orientalis* extracts exhibited antiradical activity against the 2,2-diphenyl-1-picrylhydrazyl radicals ($DPPH^{\bullet}$) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) cation radicals ($ABTS^{+\bullet}$) that were used as suitable models to detect antioxidants in food [55]. The $DPPH^{\bullet}$/$ABTS^{+\bullet}$ scavenging potentials were 42.14–48.63/45.03–53.40 μM Trolox eq./g for the wild samples, and they were slightly lower for the cultivated samples, i.e., 45.11/50.29 μM Trolox eq./g (Table 3). The antiradical activities of the strawberry extracts were as follows: *F. viridis* ($DPPH^{\bullet}$ 27.5–29.2 μM Trolox eq./g; $ABTS^{+\bullet}$ 35.1–36.2 μM Trolox eq./g), *F. vesca* ($DPPH^{\bullet}$ 15.2 μM Trolox eq./g; $ABTS^{+\bullet}$ 19.7 μM Trolox eq./g), and *F. ananassa* ($DPPH^{\bullet}$ 9.3 μM Trolox eq./g; $ABTS^{+\bullet}$ 14.7 μM Trolox eq./g) [10]. These findings indicate that *F. orientalis* fruit extracts have the highest antiradical activity.

The most active compounds of the *F. orientalis* fruit extracts were found after HPLC microfractionation coupled with bioactivity assaying by $DPPH^{\bullet}$ radicals scavenging. This was achieved by collecting HPLC eluate at 15-s intervals, solvent removal, and initiating a reaction of eluate methanol solution with $DPPH^{\bullet}$ radicals, followed by the spectrophotometric detection of the reaction progress. One-time separation of 10 mg of *F. orientalis* fruit extracts allowed us to collect 144 microfractions, of which fourteen were determined as active and included ascorbic acid, gallic acid, strictinin, lambertianin C, agrimoniin, 1-*O*-ellagoyl gentiobiose, 1-*O*-ellagoyl glucose, ellagic acid, procyanidin B2, catechin, epicatechin, cyanidin 3-*O*-glucoside, quercetin 3-*O*-arabinoside, and quercetin 3-*O*-(6″-*O*-acetyl)-glucoside (Figure 3). The results of the in vitro studies of the antiradical activity of the pure compounds confirmed the results of HPLC microfractionation (Table 4). The IC_{50} values varied from 2.37 μM (agrimoniin) to 27.19 μM (1-*O*-ellagoyl gentiobiose) in the $DPPH^{\bullet}$ scavenging assay and from 2.09 μM (agrimoniin) to 36.82 μM (1-*O*-ellagoyl gentiobiose) in the $ABTS^{+\bullet}$ scavenging assay, which was significantly superior, or similar, to the activity of reference antioxidant Trolox ($DPPH^{\bullet}$ 18.72 μM; $ABTS^{+\bullet}$ 22.57 μM). Therefore, it is clear that the *F. orientalis* fruits are a good source of antioxidant compounds.

Table 3. Antiradical activity of *F. orientalis* fruit extracts and selected compounds against 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH•) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) cation radicals ($ABTS^{+\bullet}$).

Extract	DPPH•		$ABTS^{+\bullet}$	
	μM Trolox eq./g		μM Trolox eq./g	
F. orientalis extract (wild, Yakutia)	48.63 ± 0.98^{b}		53.40 ± 1.09^{b}	
F. orientalis extract (wild, Buryatia)	42.14 ± 0.84^{a}		45.03 ± 0.90^{a}	
F. orientalis extract (cultivated)	45.11 ± 0.90^{ab}		50.29 ± 0.93^{ab}	
Compound	IC_{50}, μM	mM Trolox eq./g	IC_{50}, μM	mM Trolox eq./g
Ascorbic acid	11.35 ± 0.22^{d}	9.37 ± 0.18^{g}	10.62 ± 0.20^{ef}	10.02 ± 0.21^{h}
Gallic acid	8.03 ± 0.15^{c}	13.71 ± 0.28^{i}	7.07 ± 0.14^{d}	15.57 ± 0.31^{i}
Strictinin	2.53 ± 0.05^{a}	11.67 ± 0.23^{h}	2.11 ± 0.04^{a}	14.00 ± 0.28^{i}
Lambertianin C	3.04 ± 0.06^{b}	2.23 ± 0.04^{c}	2.73 ± 0.05^{b}	2.44 ± 0.05^{c}
Agrimoniin	2.37 ± 0.04^{a}	4.22 ± 0.08^{e}	2.09 ± 0.04^{a}	4.79 ± 0.10^{e}
1-*O*-Ellagoyl gentiobiose	27.19 ± 0.53^{i}	1.10 ± 0.02^{a}	36.82 ± 0.73^{i}	0.81 ± 0.02^{a}
1-*O*-Ellagoyl glucose	25.67 ± 0.53^{h}	1.57 ± 0.03^{b}	31.14 ± 0.63^{i}	1.29 ± 0.02^{b}
Ellagic acid	21.52 ± 0.43^{g}	2.88 ± 0.05^{d}	22.73 ± 0.49^{h}	2.73 ± 0.05^{c}
Procyanidin B2	3.57 ± 0.07^{b}	9.07 ± 0.18^{xx}	5.29 ± 0.10^{c}	6.12 ± 0.12^{f}
Catechin	11.85 ± 0.23^{d}	5.45 ± 0.10^{f}	9.41 ± 0.18^{e}	6.86 ± 0.14^{g}
Epicatechin	11.03 ± 0.22^{d}	5.85 ± 0.12^{f}	9.30 ± 0.17^{e}	6.94 ± 0.14^{g}
Cyanidin 3-*O*-glucoside	15.53 ± 0.31^{e}	2.49 ± 0.05^{d}	10.75 ± 0.17^{ef}	3.89 ± 0.08^{d}
Quercetin 3-*O*-arabinoside	10.92 ± 0.20^{d}	3.95 ± 0.08^{e}	11.63 ± 0.23^{f}	3.71 ± 0.07^{d}
Quercetin 3-*O*-(6″-*O*-acetyl)-glucoside	14.76 ± 0.29^{e}	2.51 ± 0.05^{d}	16.83 ± 0.34^{g}	2.20 ± 0.04^{c}
Trolox	18.72 ± 0.39^{f}	-	22.57 ± 0.45^{h}	-

Values with different letters (a–i) indicate statistically significant differences among groups of same method, separately for extracts and compounds at $p < 0.05$ by one-way ANOVA.

Table 4. Content of 20 phenolic compounds and ascorbic acid (mg/100 g fresh weight) and antioxidant potential (μmol trolox eq./100 g fresh weight) of *F. orientalis* fruits during storage at 4 °C (7 days) and 20 °C (3 days).

Compound		Day of Storage							
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Gallic acid	4 °C 20 °C	63 (2.5)	65 (2.6) 67 (2.7) *	69 (2.7) * 76 (3.0) *	74 (3.0) * 89 (3.6) *	79 (3.1) * n.a.	84 (3.3) * n.a.	85 (3.4) * n.a.	87 (3.4) * n.a.
Ellagitannins									
Strictinin	4 °C 20 °C	165 (6.5)	164 (6.5) 154 (6.1) *	160 (6.4) 138 (5.5) *	152 (6.0) * 107 (4.2) *	148 (5.7) * n.a.	140 (5.4) * n.a.	131 (5.2) * n.a.	118 (2.6) * n.a.
Lambertianin C	4 °C 20 °C	17 (0.8)	17 (0.7) 15 (0.6) *	16 (0.7) 13 (0.5) *	16 (0.7) 9 (0.3) *	16 (0.7) n.a.	15 (0.7) * n.a.	15 (0.7) * n.a.	14 (0.6) * n.a.
Agrimoniin	4 °C 20 °C	150 (5.8)	147 (5.8) 140 (5.2) *	143 (5.7) * 118 (4.7) *	140 (5.6) * 98 (3.9) *	132 (5.2) * n.a.	126 (5.0) * n.a.	114 (4.3) * n.a.	109 (4.3) * n.a.
1-*O*-Ellagoyl gentiobiose	4 °C 20 °C	3 (0.1)	3 (0.1) 2 (0.1) *	3 (0.1) 1 (0.0) *	2 (0.1) * 1 (0.0) *	2 (0.1) * n.a.	2 (0.1) * n.a.	1 (0.0) * n.a.	1 (0.0) * n.a.
1-*O*-Ellagoyl glucose	4 °C 20 °C	2 (0.1)	2 (0.1) 2 (0.1)	2 (0.1) 3 (0.1) *	3 (0.1) * 4 (0.2) *	3 (0.1) * n.a.	4 (0.2) * n.a.	4 (0.2) * n.a.	4 (0.2) * n.a.
Ellagic acid	4 °C 20 °C	28 (1.1)	30 (1.2) 35 (1.6) *	33 (1.4) * 49 (2.0) *	35 (1.5) * 58 (2.3) *	36 (1.5) * n.a.	39 (1.6) * n.a.	41 (1.6) * n.a.	45 (1.8) * n.a.
Subtotal	4 °C 20 °C	365	363 348	357 322	348 277	337 n.a.	326 n.a.	306 n.a.	291 n.a.
Catechins and procyanidins									
Procyanidin B2	4 °C 20 °C	7 (0.3)	7 (0.3) 6 (0.3) *	7 (0.3) 6 (0.3) *	6 (0.3) * 4 (0.2) *	6 (0.3) * n.a.	5 (0.2) * n.a.	5 (0.2) * n.a.	5 (0.2) * n.a.
Catechin	4 °C 20 °C	3 (0.1)	3 (0.1) 2 (0.1) *	3 (0.1) 1 (0.0) *	2 (0.1) * 1 (0.0) *	2 (0.1) * n.a.	2 (0.1) * n.a.	2 (0.1) * n.a.	2 (0.1) * n.a.
Epicatechin	4 °C 20 °C	3 (0.1)	3 (0.1) 2 (0.1) *	3 (0.1) 1 (0.0) *	2 (0.1) * 0	2 (0.1) * n.a.	2 (0.1) * n.a.	2 (0.1) * n.a.	1 (0.0) * n.a.
Subtotal	4 °C 20 °C	13	13 10	13 8	10 5	10 n.a.	9 n.a.	9 n.a.	8 n.a.

Table 4. *Cont.*

Compound		Day of Storage							
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Anthocyanidins									
Cyanidin 3-*O*-glucoside	4 °C 20 °C	3 (0.1)	3 (0.1) 3 (0.1)	3 (0.1) 2 (0.1) *	3 (0.1) 2 (0.1) *	2 (0.1) * n.a.	2 (0.1) * n.a.	2 (0.1) * n.a.	2 (0.1) * n.a.
Pelargonidin 3-*O*-glucoside	4 °C 20 °C	8 (0.3)	8 (0.3) 7 (0.3) *	8 (0.3) 6 (0.2) *	7 (0.3) * 6 (0.2) *	7 (0.3) * n.a.	7 (0.3) * n.a.	6 (0.2) * n.a.	6 (0.2) * n.a.
Pelargonidin *O*-malonyl-hexoside	4 °C 20 °C	2 (0.1)	2 (0.1) 1 (0.0) *	2 (0.1) 1 (0.0) *	2 (0.1) 0	1 (0.0) * n.a.	1 (0.0) * n.a.	1 (0.0) * n.a.	1 (0.0) * n.a.
Pelargonidin di-*O*-malonyl-hexoside	4 °C 20 °C	4 (0.2)	4 (0.2) 3 (0.2)	4 (0.2) 1 (0.1) *	3 (0.2) * 1 (0.1) *	3 (0.2) * n.a.	3 (0.2) * n.a.	2 (0.1) * n.a.	2 (0.1) * n.a.
Subtotal	4 °C 20 °C	17	17 14	17 10	15 9	13 n.a.	13 n.a.	11 n.a.	11 n.a.
Flavonoids									
Kaempferol 3-*O*-rutinoside	4 °C 20 °C	2 (0.1)	2 (0.1) 2 (0.1)	2 (0.1) 2 (0.1)	2 (0.1) 1 (0.0) *	2 (0.1) n.a.	2 (0.1) n.a.	2 (0.1) n.a.	2 (0.1) n.a.
Kaempferol 3-*O*-glucoside	4 °C 20 °C	2 (0.1)	2 (0.1) 2 (0.1)	2 (0.1) 2 (0.1)	2 (0.1) 2 (0.0)	2 (0.1) n.a.	2 (0.1) n.a.	2 (0.1) n.a.	2 (0.1) n.a.
Quercetin 3-*O*-arabinoside	4 °C 20 °C	2 (0.1)	2 (0.1) 2 (0.1)	2 (0.1) 2 (0.1)	2 (0.1) 2 (0.0)	2 (0.1) n.a.	2 (0.1) n.a.	2 (0.1) n.a.	2 (0.1) n.a.
Quercetin 3-*O*-(6″-*O*-acetyl)-glucoside	4 °C 20 °C	2 (0.1)	2 (0.1) 2 (0.1)	2 (0.1) 1 (0.0) *	1 (0.0) * 1 (0.0) *	1 (0.0) * n.a.	1 (0.0) * n.a.	1 (0.0) * n.a.	1 (0.0) * n.a.
Kaempferol 3-*O*-(6″-*O*-*p*-coumaroyl)-glucoside	4 °C 20 °C	1 (0.0)	1 (0.0) 1 (0.0)	1 (0.0) 1 (0.0)	1 (0.0) 0	1 (0.0) n.a.	1 (0.0) n.a.	1 (0.0) n.a.	1 (0.0) n.a.
Quercetin *O*-acetyl-*O*-malonyl-*O*-hexoside	4 °C 20 °C	2 (0.1)	2 (0.1) 2 (0.1)	2 (0.1) 0	1 (0.0) * 0	1 (0.0) * n.a.	1 (0.0) * n.a.	1 (0.0) * n.a.	1 (0.0) * n.a.
Subtotal	4 °C 20 °C	11	11 11	11 8	9 6	9 n.a.	9 n.a.	9 n.a.	9 n.a.
Total phenolic compounds	4 °C 20 °C	469	469 450	467 424	456 386	448 n.a.	441 n.a.	420 n.a.	406 n.a.
Ascorbic acid	4 °C 20 °C	145 (5.8)	143 (5.7) 130 (5.2) *	140 (5.6) 106 (4.1) *	137 (5.2) * 73 (2.9) *	130 (5.0) * n.a.	124 (4.8) * n.a.	110 (4.3) * n.a.	98 (3.9) * n.a.
Total antioxidant potential	4 °C 20 °C	497 (19.8)	487 (19.2) 432 (17.1) *	472 (18.6) 360 (14.4) *	457 (18.0) * 253 (10.1) *	440 (17.2) * n.a.	412 (16.5) * n.a.	379 (15.0) * n.a.	326 (12.8) * n.a.

n.a.—not analyzed. The zero content means not detected. Asterisk indicates significant difference ($p < 0.05$) vs. initial level (0 days).

3.5. Variation of Phenolic Compounds, Ascorbic Acid and Antioxidant Potential of F. orientalis Fruits during Postharvest Storage

The proper storage of fruits is an important problem of the food industry and involves the selection of the optimal conditions for the best preservation of the appearance (texture), nutrients, and beneficial metabolites that give the products valuable functional properties. The complexity of this problem for strawberries lies in their short shelf life, owing to the high moisture content in the fruit pulp and the high carbohydrate concentration, which leads to rapid fermentation and souring of the product. Fruits of *F. orientalis* are not an exception, and the problem of their postharvest storage is relevant. In this study, we investigated the changes that occur in *F. orientalis* fruits during three types of storage, including (1) the storage of fresh berries at cold and room temperatures, (2) the storage of fresh frozen berries and the effect of thawing on this process, and (3) the storage of dried berries at cold and room temperatures.

3.5.1. Storage of Fresh *F. orientalis* Fruits at Cold and Room Temperatures

The periods of postharvest storage for the fresh fruits were chosen as 7 days at cold temperature (4 °C) and 3 days at room temperature (20 °C), primarily owing to the loss of good appearance and acceptable taste. The cold temperature storage resulted in a gradual loss of ascorbic acid by 32% (145→98 mg/100 g) and of all phenolic groups, such as ellagitannins by 20% (365→291 mg/100 g), catechins and procyanidins by 39% (13→8 mg/100 g), anthocyanidins by 35% (17→11 mg/100 g), and flavonoids by 18% (11→9 mg/100 g) (Table 4). Only gallic acid and ellagic acid contents increased by 38% (63→87 mg/100 g) and 61% (28→45 mg/100 g), respectively; however, at the same time, the total phenolic content loss in *F. orientalis* fruits during cold temperature storage was 13% (469→406 mg/100 g). When

stored at room temperature, a more rapid decline in the levels of ascorbic acid (50% loss; 145→73 mg/100 g), ellagitannins (24% loss; 365→277 mg/100 g), catechins and procyanidins (62% loss; 13→5 mg/100 g), anthocyanidins (47% loss; 17→9 mg/100 g), and flavonoids (46% loss; 11→6 mg/100 g) in strawberry fruits was detected. At the same time, the concentrations of gallic acid (63→89 mg/100 g) and ellagic acid (28→58 mg/100 g) were higher at the end of the storage period.

By monitoring the changes in metabolite content, we determined that high ascorbic acid loss in *F. orientalis* fruits was not surprising, owing to the compound instability in water media, such as fruit pulp [56], and previous studies repeatedly demonstrated a decrease in ascorbic acid content in *F. ananassa* fruits during cold storage of up to 50% and higher [57,58]. Similar results were obtained for unstable anthocyanidins, which decompose to aglycones in strawberry pulp after hydrolytic or enzymatic cleavage [59]. Ellagitannins represent another unstable phenolic group, which disintegrates via the water, acids, and enzymes that are present in fruit pulp [60]. This fact was clearly confirmed by the increased content of gallic and ellagic acids released after ellagitannins hydrolysis. These phenomena were previously observed in the fruits of *F. ananassa* [61] and *F. viridis* [10] and are apparently common for strawberries during postharvest storage at cold and room temperatures. These processes are responsible for the decrease in the total antioxidant potential of the *F. orientalis* fruits (497→326 μmol trolox eq./100 g at 4 °C; 497→253 μmol trolox eq./100 g at 20 °C), owing to the declining content of phenolics and ascorbic acid, which are the main antioxidants. Thus, the obtained results suggest that the storage of *F. orientalis* fresh fruits at cold temperature (4 °C) allows us to retain phenolics, ascorbic acid, and antioxidant potential for a longer period of time.

3.5.2. Storage of Frozen *F. orientalis* Fruits: Influence of Thawing

Freezing, as a technological process that is used in the food industry, allows us to preserve fresh fruit for a longer period of time. The main problem is the preliminary thawing (defrosting) of fruits, after which the cellular structure is impaired, owing to the formation of ice crystals. In most cases, thawing adversely affects the appearance of fruits and results in the loss of the initial shape, and it changes the chemical composition, owing to the influence of the enzymes released from the destroyed cells [62]. Hydrolytically and enzymatically unstable substances can change dramatically or completely disappear. It is known that the content of metabolites in frozen strawberries (e.g., ascorbic acid [63] or anthocyanins and other phenolics in *F. ananassa* [64] or phenolics in *F. vesca* [62]) can be reduced to trace or zero levels after thawing. In this study, we performed a comparative quantitative analysis of the phenolic compounds, ascorbic acid, and antioxidant potential of *F. orientalis* fruits stored at −20 °C for 4 years, before and after 30 min of thawing.

During the four-year experimental period, the total phenolic content in frozen fruits, before thawing, decreased by 7% (1st year), 18% (2nd year), 31% (3rd year), and 45% (4th year) from the initial level; after 1 year of storage, the loss of concentration for various compounds was insignificant (Table 5).

At the end of the first year, even ascorbic acid (a light and oxygen sensitive compound) showed 94% safety. The thawing step resulted in even greater reductions of total phenolic contents of 46% (1st year), 50% (2nd year), 54% (3rd year), and 54% (4th year) from the initial level. Ascorbic acid lost 81% and 88% of its contents after the first and second year of storage, respectively, and became undetectable after longer periods of storage. The devastating impact of thawing was detected for ellagitannins, ellagic acid glycosides, catechins, anthocyanins, and acylated flavonols; the concentrations of these compounds decreased by over 90%, compared to their starting levels. In addition, there were accumulations of gallic (48% profit; 65→96 mg/100 g) and ellagic acids (259% profit; 29→104 mg/100 g) caused by the disintegration of ellagitannins.

Table 5. Content of 20 phenolic compounds and ascorbic acid (mg/100 g fresh weight) and antioxidant potential (μmol trolox eq./100 g fresh weight) of *F. orientalis* fruits during storage at −20 °C (4 years), before (B) and after (A) thawing.

Compound	Variant	Year of Storage				
		0	**1**	**2**	**3**	**4**
Gallic acid	B	65 (1.9)	60 (1.8) *	52 (1.5) *	43 (1.2) *	24 (0.7) *
	A		73 (2.1) *	89 (2.6) *	94 (2.8) *	96 (2.8) *
Ellagitannins						
Strictinin	B	167 (5.0)	160 (4.8) *	143 (4.3) *	129 (3.8) *	110 (3.3) *
	A		48 (1.4) *	25 (0.7) *	19 (0.5) *	7 (0.2) *
Lambertianin C	B	17 (0.5)	14 (0.4) *	10 (0.3) *	7 (0.2) *	2 (0.1) *
	A		2 (0.1) *	0	0	0
Agrimoniin	B	148 (4.7)	135 (4.0) *	124 (3.7) *	106 (3.1) *	88 (2.6) *
	A		24 (0.7) *	17 (0.5) *	2 (0.1)	0
1-*O*-Ellagoyl gentiobiose	B	3 (0.1)	2 (0.1) *	1 (0.0) *	0	0
	A		0	0	0	0
1-*O*-Ellagoyl glucose	B	2 (0.1)	2 (0.1)	2 (0.1)	0	0
	A		4 (0.1) *	5 (0.2) *	4 (0.1) *	2 (0.1)
Ellagic acid	B	29 (0.9)	27 (0.8)	23 (0.6) *	20 (0.7) *	18 (0.5) *
	A		87 (2.6) *	92 (2.7) *	95 (2.9) *	104 (3.3) *
Subtotal	B	366	340	303	262	218
	A		165	139	120	113
Catechins and procyanidins						
Procyanidin B2	B	7 (0.2)	7 (0.2)	7 (0.2)	6 (0.2) *	6 (0.1) *
	A		5 (0.1) *	5 (0.1) *	2 (0.0) *	1 (0.0) *
Catechin	B	4 (0.1)	3 (0.1) *	2 (0.1) *	1 (0.0) *	1 (0.0) *
	A		0	0	0	0
Epicatechin	B	3 (0.1)	2 (0.1) *	1 (0.0) *	0	0
	A		0	0	0	0
Subtotal	B	14	12	10	7	7
	A		5	5	2	1
Anthocyanidins						
Cyanidin 3-*O*-glucoside	B	2 (0.1)	2 (0.1)	1 (0.0) *	1 (0.0) *	1 (0.0) *
	A		1 (0.0)	0	0	0
Pelargonidin 3-*O*-glucoside	B	9 (0.3)	9 (0.3)	7 (0.2) *	6 (0.2) *	4 (0.1) *
	A		1 (0.0)	0	0	0
Pelargonidin *O*-malonyl-hexoside	B	1 (0.0)	1 (0.0)	0	0	0
	A		0	0	0	0
Pelargonidin di-*O*-malonyl-hexoside	B	4 (0.1)	4 (0.1)	2 (0.1) *	0	0
	A		0	0	0	0
Subtotal	B	16	16	10	7	5
	A		2	0	0	0

Table 5. *Cont.*

Compound	Variant	Year of Storage				
		0	**1**	**2**	**3**	**4**
Flavonoids						
Kaempferol 3-*O*-rutinoside	B A	2 (0.1)	2 (0.1) 2 (0.1) *	2 (0.1) 1 (0.0) *	2 (0.1) 1 (0.0) *	2 (0.1) 1 (0.0) *
Kaempferol 3-*O*-glucoside	B A	2 (0.1)	2 (0.1) 2 (0.1)	2 (0.1) 2 (0.1)	2 (0.1) 3 (0.1) *	2 (0.1) 3 (0.1) *
Quercetin 3-*O*-arabinoside	B A	2 (0.1)	2 (0.1) 1 (0.0) *	2 (0.1) 1 (0.0) *	1 (0.0) * 0	1 (0.0) * 0
Quercetin 3-*O*-(6″-*O*-acetyl)-glucoside	B A	1 (0.0)	1 (0.0) 0	1 (0.0) 0	0 0	0 0
Kaempferol 3-*O*-(6″-*O*-*p*-coumaroyl)-glucoside	B A	1 (0.0)	1 (0.0) 0	1 (0.0) 0	1 (0.0) 0	0 0
Quercetin *O*-acetyl-*O*-malonyl-*O*-hexoside	B A	2 (0.1)	2 (0.1) 0	2 (0.1) 0	1 (0.0) * 0	1 (0.0) * 0
Subtotal	B A	10	10 5	10 4	7 4	5 4
Total phenolic compounds	B A	471	438 250	385 237	326 220	259 214
Ascorbic acid	B A	145 (4.3)	136 (4.1) * 27 (0.8) *	124 (3.7) * 18 (0.5) *	109 (3.5) * 0	83 (2.4) * 0
Total antioxidant potential	B A	483 (19.3)	454 (18.1) 198 (7.9) *	396 (15.8) * 145 (5.7) *	354 (14.1) * 110 (4.0) *	193 (7.7) * 96 (3.5) *

The zero content means not detected. Asterisk indicates significant difference ($p < 0.05$) vs. initial level (0 days).

Thus, the freezing of *F. orientalis* fruits, as a way of postharvest storage, allows us to preserve the phenolic compounds and ascorbic acid for a long period of time; however, thawing negates all advantages of freezing. The metabolic changes in the *F. orientalis* fruits were primarily reflected by the decrease in antioxidant potential when using thawing (483→96 µmol trolox eq./100 g). Therefore, freezing is a valid method of strawberry preservation; however, to reduce losses of valuable substances and bioactivity, the post-freezing treatment of fruits must be excluded or reduced.

3.5.3. Storage of Freeze-dried *F. orientalis* Fruits at Cold and Room Temperature

Drying, as a method of post-harvest fruit preservation, is often used for cultivated [65] and wild strawberry storage [66]. The preliminary study on the nutritional and chemical composition of *F. orientalis* fruits, dried using four different methods (i.e., sun-drying, heat-drying, microwave-drying, and freeze-drying), allowed us to choose freeze-drying as the best method for preserving fruits, owing to the maximum preservation of nutrients and phytochemicals (Table 6). Drying has the least impact on the nutrients, titratable acids, flavonoids, and total phenolic contents. The content of ascorbic acid was significantly different in freeze-dried fruits (853.1 mg/100 g), compared to the fruits preserved by sun-drying (89% loss; 91.6 mg/100 g), heat-drying (82% loss; 153.5 mg/100 g), and microwave-drying (35% loss; 554.4 mg/100 g). Sun-dried samples demonstrated the worst results and showed an 87% loss of anthocyanins, 83% loss of catechins, and 91% loss of procyanidins, compared to freeze-dried fruits. However, an 82% increase in ellagic acid was detected in sun-dried *F. orientalis* fruits, which is probably due to the degradation of ellagitannin. Therefore, freeze-dried fruits of *F. orientalis* were used to study storage stability of phenolic compounds, ascorbic acid, and antioxidant potential storage at 4 °C and 20 °C for a 4-year period.

Table 6. Nutritional and chemical composition of dried *F. orientalis* fruits (± S.D.).

Parameter	Sun-Dried	Heat-Dried	Microwave-Dried	Freeze-Dried
Ash, g/100 g	5.9 ± 0.2 ^{a}	5.9 ± 0.2 ^{a}	5.9 ± 0.2 ^{a}	5.9 ± 0.2 ^{a}
Protein, g/100 g	4.9 ± 0.1 ^{a}	5.0 ± 0.1 ^{a}	5.0 ± 0.1 ^{a}	4.9 ± 0.1 ^{a}
Lipids, g/100 g	5.6 ± 0.2 ^{a}	5.6 ± 0.2 ^{a}	5.8 ± 0.2 ^{a}	5.8 ± 0.2 ^{a}
Carbohydrates, g/100 g	64.8 ± 1.9 ^{a}	65.2 ± 2.1 ^{a}	64.3 ± 1.8 ^{a}	64.5 ± 1.9 ^{a}
Total dietary fibers, g/100 g	27.6 ± 0.6 ^{a}	27.9 ± 0.6 ^{a}	28.0 ± 0.6 ^{a}	28.3 ± 0.6 ^{a}
Titratable acids, g/100 g	8.1 ± 0.2 ^{a}	8.0 ± 0.2 ^{a}	8.3 ± 0.2 ^{a}	8.3 ± 0.2 ^{a}
Ascorbic acid, mg/100 g	91.6 ± 1.8 ^{a}	153.5 ± 3.1 ^{b}	554.4 ± 11.0 ^{c}	853.1 ± 17.0 ^{d}
Anthocyanins, mg/100 g	10.4 ± 0.2 ^{a}	44.2 ± 0.9 ^{b}	75.3 ± 1.5 ^{c}	82.4 ± 1.6 ^{d}
Ellagic acid free, mg/100 g	573.0 ± 11.5 ^{c}	453.1 ± 9.1 ^{b}	330.6 ± 6.7 ^{a}	314.2 ± 6.2 ^{a}
Ellagic acid total, mg/100 g	1429.6 ± 28.5 ^{a}	1510.6 ± 30.0 ^{bc}	1522.1 ± 30.4 ^{c}	1536.1 ± 30.6 ^{c}
Catechins, mg/100 g	7.2 ± 0.1 ^{a}	31.7 ± 0.6 ^{b}	40.9 ± 0.8 ^{c}	42.4 ± 0.8 ^{c}
Procyanidins, mg/100 g	5.1 ± 0.1 ^{a}	31.2 ± 0.6 ^{b}	52.1 ± 1.0 ^{c}	56.3 ± 1.1 ^{c}
Flavonoids, mg/100 g	67.2 ± 1.4 ^{a}	67.1 ± 1.4 ^{a}	67.0 ± 1.4 ^{a}	67.5 ± 1.4 ^{a}
Total phenolics, g/100 g	2.6 ± 0.1 ^{a}	2.9 ± 0.1 ^{ab}	3.4 ± 0.1 ^{b}	3.5 ± 0.1 ^{b}

Values with different letters (a–d) indicate statistically significant differences among groups at $p < 0.05$ by one-way ANOVA.

Cold-temperature storage promotes the better preservation of phenolic compounds and ascorbic acid in the freeze-dried fruits of *F. orientalis*: the final losses (at the end of the 4th year) of contents were 2% and 6%, respectively (Table 7). Room-temperature storage resulted in a greater decrease in the levels of phenolic compounds and ascorbic acid, i.e., 5% and 10%, respectively. The most sensitive compounds were anthocyanins, with total reductions of 18% at 4 °C and 33% at 20 °C. Only gallic and ellagic acids demonstrated slowly increasing contents, which were more pronounced for cold-temperature storage than for room-temperature storage. The total antioxidant content decreased from 3352 µmol trolox eq./100 g dry weight to 3117 µmol trolox eq./100 g dry weight at 4 °C (7% loss) and to 2949 µmol trolox eq./100 g dry weight at 20 °C (12% loss). Our findings indicate that the freeze-drying of *F. orientalis* fruits, followed by cold-temperature storage, is a gentler method of strawberry preservation, compared to the storage of frozen fruits.

It has been previously reported that the freeze-drying of garden strawberries (*F. ananassa*) and the use of low processing temperatures improves the sensory quality of dried fruits [67] and preserves their volatile compounds [65]. A low variation of total phenolics and antiradical activity was found after the freeze-drying of *F. ananassa* var. Camarosa, while the anthocyanin content varied remarkably [68]. The advantages of cold-temperature storage were previously noted for freeze-dried pears (*Pyrus communis*), which resulted in the superior retention of ascorbic acid, vitamin E, total polyphenols, and antioxidant activity in 12-month cold-stored fruits [69]. The combination of freeze-drying and cold storage for 12 weeks of pomegranate fruits resulted in the high stability of total phenolics, titratable acids, radical-scavenging activity, and ferric ion reducing antioxidant power [70].

Based on the previous findings and the results in this study on the post-harvest stability of metabolites and bioactivity of *F. orientalis*, it can be argued that cold storage is the preferred storage method for fresh and freeze-dried fruits. These conditions allow us to significantly reduce the losses of chemicals, thus preserving the biological activity of foodstuff. Freezing and thawing of strawberry fruits have the greatest adverse effects and lead to large losses of labile compounds and a reduced antioxidant effect.

Table 7. Content of 20 phenolic compounds and ascorbic acid (mg/100 g dry weight) and antioxidant potential (μmol trolox eq./100 g dry weight) of freeze-dried *F. orientalis* fruits during 4-year storage at 4 °C and 20 °C.

Compound	Variant	Year of Storage				
		0	1	2	3	4
Gallic acid	4 °C	468 (18.7)	468 (18.5)	472 (18.8)	475 (18.7)	483 (19.3)
	20 °C		472 (18.9)	489 (19.2)	501 (20.0) *	506 (20.6) *
Ellagitannins						
Strictinin	4 °C	1052 (42.0)	1043 (41.7)	1022 (40.7)	1008 (39.9)	995 (39.6)
	20 °C		1023 (40.9)	1002 (39.8)	983 (39.3) *	967 (38.2) *
Lambertianin C	4 °C	104 (4.1)	101 (4.0)	99 (3.9)	97 (3.8)	95 (3.8) *
	20 °C		95 (3.8) *	92 (3.6) *	88 (3.5) *	82 (3.2) *
Agrimoniin	4 °C	959 (38.4)	950 (38.1)	943 (37.7)	936 (37.4)	930 (37.2)
	20 °C		935 (37.4)	920 (36.8)	909 (36.0) *	886 (35.4) *
1-*O*-Ellagoyl gentiobiose	4 °C	23 (0.9)	23 (0.9)	22 (0.8)	22 (0.8)	20 (0.7) *
	20 °C		22 (0.8)	20 (0.7) *	18 (0.7) *	15 (0.5) *
1-*O*-Ellagoyl glucose	4 °C	16 (0.6)	16 (0.5)	16 (0.5)	17 (0.6)	17 (0.7)
	20 °C		16 (0.5)	17 (0.6)	17 (0.6)	17 (0.6)
Ellagic acid	4 °C	314 (12.5)	314 (12.7)	317 (12.8)	328 (13.1)	352 (14.0) *
	20 °C		320 (12.9)	347 (13.7) *	361 (14.3) *	379 (15.1) *
Subtotal	4 °C	2468	2447	2419	2408	2409
	20 °C		2411	2398	2331	2346
Catechins and procyanidins						
Procyanidin B2	4 °C	54 (2.0)	52 (2.0)	52 (2.0)	50 (1.9) *	48 (1.8) *
	20 °C		51 (2.1) *	48 (1.7) *	47 (1.7) *	43 (1.6) *
Catechin	4 °C	28 (1.1)	27 (1.0)	25 (0.9) *	25 (0.9) *	24 (0.9) *
	20 °C		25 (0.9) *	23 (0.9) *	23 (0.9) *	20 (0.7) *
Epicatechin	4 °C	20 (0.8)	18 (0.6)	18 (0.6)	17 (0.6) *	17 (0.5) *
	20 °C		16 (0.5) *	16 (0.5) *	14 (0.5) *	12 (0.4) *
Subtotal	4 °C	102	97	95	92	89
	20 °C		92	87	84	75
Anthocyanidins						
Cyanidin 3-*O*-glucoside	4 °C	11 (0.4)	11 (0.5)	10 (0.4)	10 (0.4) *	9 (0.3) *
	20 °C		10 (0.4) *	10 (0.4) *	9 (0.3) *	9 (0.3) *
Pelargonidin 3-*O*-glucoside	4 °C	42 (1.6)	40 (1.5)	39 (1.4) *	37 (1.2) *	35 (1.0) *
	20 °C		35 (1.0) *	32 (0.8) *	30 (0.7) *	28 (0.6) *
Pelargonidin *O*-malonyl-hexoside	4 °C	5 (0.2)	5 (0.2)	5 (0.2)	4 (0.2) *	4 (0.2) *
	20 °C		4 (0.2) *	4 (0.2) *	3 (0.2) *	3 (0.2) *
Pelargonidin di-*O*-malonyl-hexoside	4 °C	19 (0.7)	19 (0.7)	17 (0.5) *	17 (0.5) *	15 (0.4) *
	20 °C		17 (0.5) *	17 (0.5) *	15 (0.4) *	12 (0.4) *
Subtotal	4 °C	77	75	71	68	63
	20 °C		66	63	57	52

Table 7. *Cont.*

Compound	Variant	Year of Storage				
		0	1	2	3	4
Flavonoids						
Kaempferol 3-*O*-rutinoside	4 °C 20 °C	17 (0.7)	17 (0.7) 17 (0.7)	17 (0.7) 16 (0.7)	17 (0.6) 16 (0.6)	16 (0.7) 16 (0.6)
Kaempferol 3-*O*-glucoside	4 °C 20 °C	19 (0.8)	19 (0.8) 19 (0.8)	19 (0.7) 19 (0.8)	18 (0.8) 20 (0.9)	19 (0.8) 20 (0.9)
Quercetin 3-*O*-arabinoside	4 °C 20 °C	15 (0.6)	15 (0.6) 15 (0.6)	14 (0.6) 14 (0.6)	14 (0.5) 14 (0.6)	14 (0.5) 13 (0.5) *
Quercetin 3-*O*-(6″-*O*-acetyl)-glucoside	4 °C 20 °C	7 (0.3)	6 (0.3) 6 (0.2)	5 (0.2) * 6 (0.2)	5 (0.2) * 5 (0.2) *	5 (0.1) * 5 (0.2) *
Kaempferol 3-*O*-(6″-*O*-*p*-coumaroyl)-glucoside	4 °C 20 °C	8 (0.3)	8 (0.3) 7 (0.3) *	8 (0.3) 7 (0.3) *	7 (0.3) * 7 (0.3) *	7 (0.3) * 5 (0.2) *
Quercetin *O*-acetyl-*O*-malonyl-*O*-hexoside	4 °C 20 °C	12 (0.5)	12 (0.5) 10 (0.4) *	11 (0.4) * 10 (0.2) *	11 (0.3) * 10 (0.2) *	10 (0.3) * 9 (0.0) *
Subtotal	4 °C 20 °C	78	77 74	74 72	72 72	71 68
Total phenolic compounds	4 °C 20 °C	3193	3164 3115	3131 3109	3115 3045	3115 3047
Ascorbic acid	4 °C 20 °C	848 (33.9)	835 (33.4) 826 (32.8)	824 (32.9) 803 (32.0)	810 (32.1) 785 (31.4) *	801 (32.0) 763 (30.1) *
Total antioxidant potential	4 °C 20 °C	3352 (98.2)	3301 (99.0) 3278 (98.2)	3268 (97.2) 3150 (94.5) *	3217 (96.2) * 3067 (92.0) *	3117 (95.0) * 2949 (99.5) *

The zero content means not detected. Asterisk indicates significant difference ($p < 0.05$) vs. initial level (0 days).

4. Conclusions

In this study, we first identified the metabolites of *F. orientalis* fruits using the LC–MS technique and quantified the major phenolic compounds and ascorbic acid. The oriental strawberry was determined to be a source of phenolic antioxidants, such as ellagitannins and gallic acid. This is an important point of food plant metabolomics because it is necessary for the authentication of new strawberries and strawberry-based food products. The high radical-scavenging activity of the *F. orientalis* fruit extract was a direct consequence of the high level of phenolics with confirmed antioxidant potential.

Traditionally, ripe strawberries are used unprocessed, but the consumer and biochemical qualities of fresh fruits rapidly degrade, owing to fast rotting. The most commonly used preservation techniques include the cold storage and freezing of fresh fruits, as well as various drying methods. This study evaluated the effects of cold storage on the levels of phenolic compounds and ascorbic acid, as well as the antioxidant potential in the fresh fruits of *F. orientalis* during short-term storage and in frozen fruits during long-term storage. The 7-day storage of fresh fruits at 4 °C has lower degradation effects on ascorbic acid, ellagitannins, anthocyanins, catechins, proanthocyanins, and flavonoids than room-temperature storage. Frozen fruits demonstrated good preservation of studied metabolites after 4-year storage at −20 °C; however, thawing drastically reduced the content of most compounds, as well as the antioxidant potential. With the general decline in phenolics and ascorbic acid content, there was an increase in the concentrations of gallic and ellagic acids, which was likely due to ellagitannins degradation. In contrast, compared to fresh and frozen fruits, the freeze-dried fruits of *F. orientalis* showed higher stability of labile compounds and higher antioxidant potential during 4-year cold storage. The new data provided by our study will help with the introduction of new strawberry crops and an estimation of the relevance of *F. orientalis* consumption on human health.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae8100975/s1: Table S1: *Fragaria* fruit sample description; Table S2: Reference standards used for the qualitative and quantitative analysis by HPLC-DAD-ESI-tQ-MS assays; Table S3: Regression equations, correlation coefficients (r^2), standard deviation (S_{YX}), limits of detection (LOD), limits of quantification (LOQ) and linear ranges for 17 reference standards; Table S4: Nutritional and chemical composition of wild and cultivated samples of *F. orientalis* fruits; Table S5: Demographic information of participants of *Fragaria* fruits sensory evaluation; Table S6: Sensory evaluation data of *Fragaria* fruits; Table S7: Chromatographic (*t*), ultraviolet (UV) and mass-spectrometric data of compounds **1**–**65** found in *F. orientalis* fruits; Table S8: Quantitative content of 21 compounds in *Fragaria* fruits.

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