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Maximizing the Recovery of Phenolic Antioxidants from Wild Strawberry (*Fragaria vesca*) Leaves Using Microwave-Assisted Extraction and Accelerated Solvent Extraction

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Abstract: Due to the presence of diverse phenolic classes in wild strawberry (*Fragaria vesca* L.) leaves, there is an ever-growing effort to find new, efficient methods for their recovery and detailed characterization. Microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE) were evaluated to understand the impact of the extraction temperature, extraction time, and solvent-to-sample ratio (SSR) on the quantitative and qualitative properties of the obtained extracts. The highest total phenolic content (8027 mg GA/100 g DW), as well as the highest DPPH$^{\bullet}$ antiradical activity (903 µmol TE/g DW), was obtained with ASE at 150 °C with a static time of 5 min and an SSR of 40:1, while the highest ABTS$^{\bullet+}$ antiradical activity (681 µmol TE/g DW) and FRAP (2389 µmol TE/g DW) were obtained with MAE after 5 min at 80 °C and an SSR of 40:1. A total of 54 different phenolics were identified by UPLC/MS-MS, some for the first time. The MAE extract had a higher content of phenolic acids (40%; esp. *p*-hydroxybenzoic acid, gallic acid) and myricetin, while the ASE extract was richer in proanthocyanidins (88%; esp. procyanidin B1, procyanidin trimer), flavonols (29%; esp. quercetin, quercetin-3-glucuronide, rutin), flavan-3-ols (50%; esp. epicatechin), and flavones (39%; esp. luteolin). The results indicated that for optimal extraction conditions, the target phenolics and the desired antioxidant properties of the obtained extracts should be considered.

Keywords: wild strawberry leaves; microwave-assisted extraction; accelerated solvent extraction; phenolic profile; antioxidant activity

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1. Introduction

Fragaria vesca L., commonly known as wild strawberry, is an herbaceous perennial plant from the Rosaceae family. It is widespread throughout Europe and the rest of the world, where it occurs in forests, on slopes, and on roadsides. Although wild strawberry is best known for its small aromatic fruits, its leaves are collected in the wild during the flowering season for domestic use—externally as an antiseptic, anti-inflammatory, and skin-protecting agent and internally for respiratory, gastrointestinal, and urinary disorders. There have also been reports of the leaves being used for the treatment of diabetes, cancer, and cardiovascular diseases [1–5]. As previously demonstrated [2], wild strawberry leaves are an important source of ellagitannins, proanthocyanidins, quercetin, and kaempferol glucuronide derivatives. More precisely, procyanidin B1, B2, and C1, pyrocyanidin B1, epigallocatechin, (+)-catechin, (−)-epicatechin, astringin, epicatechin-3-gallate, piceid, quercetin, quercetin-4′-glucoside, gallic acid monohydrate, kaempferol 3-*β*-d-glucopyranoside, and *trans*-resveratrol were identified in the extracts of wild strawberry leaves [4,6]. A strong to moderate correlation between the antioxidant capacity evaluated by

different methods and the presence of phenolics was demonstrated for the in vitro-cultured vegetative parts of *F. vesca* [7]. However, scientific reports validating the antioxidant properties of *F. vesca* leaves are still scarce. The extracts were mostly obtained conventionally using boiling water [4], hot water [8,9], or an aqueous alcohol solution [2,10,11] as a solvent. The prepared phenolic extracts comprised 5 [8], 10 [4], 11 [10], 20 [2], and 27 [11] identified phenolic compounds. As shown by the example of strawberry, blackcurrant, and raspberry leaves [8], not all of the compounds extracted with organic solvents (benzene, chloroform, acetone, methanol, ethanol) could be identified in plant water extracts. Due to the presence of a great diversity of phenolics, the growing demand for wild strawberry leaf extracts with additional functional properties requires innovation in ways to recover bioactive compounds that can be further applied in the pharmaceutical or food industry. Conventional extraction methods produce many hazardous residues and are very time-consuming and costly. Among the new approaches to the utilization of bioactives from fruit waste, green extraction processes are attracting a lot of attention nowadays, as they can meet requirements from environmental and economic points of view [12]. As representatives of advanced green extraction techniques, microwave-assisted (MAE) and accelerated solvent extraction (ASE) are widely applied. The principle of MAE involves the conversion of electromagnetic energy into thermal energy, which causes a pressure build-up within the cellular matrix, opening the cellular structure and resulting in the formation of pores and the release of inter- and intracellular contents. The disruption of weak hydrogen bonds is caused by the dipole rotation of the molecules with the release of heat [13]. During ASE or pressurized liquid extraction, solid matrices are dissolved by solvents at temperatures above their boiling point, with pressurization enabling them to remain in a liquid state. Sample extraction with green solvents under high pressure (4–20 MPa) and moderate to high temperatures (50–250 °C) results in the breaking of secondary bonds and thus in the acceleration of desorption and the solubilization of matrix-bound species [14]. In contrast to the dynamic mode, where fresh solvent flows continuously through the sample, in the static mode, the sample is extracted with a solvent until equilibrium is reached, followed by the collection of the analytes through rapid flushing with the solvent and compressed gas [13]. Regardless of the applied method, the extraction parameters must be carefully selected to avoid uncontrolled changes in native structures and the co-elution of unwanted compounds. To maximize the yield, an optimal ratio between the solubilization and degradation of the target molecules must be determined empirically [14].

To the best of our knowledge, MAE and ASE have not been previously tested for the extraction of phenolics from wild strawberry leaves. Hence, the aim of this study was to prepare an antioxidant-rich green extract from *F. vesca* leaves, to fully characterize its phenolic profile, and to examine the effects of the extraction temperature, time, and solvent-to-sample ratio (SSR) on the total phenolic content (TPC) and antioxidant properties of the obtained extracts in order to improve the extraction efficiency along with the sustainable use of energy and materials. Knowing the exact phenolic profile of wild strawberry leaves could further improve their application for food fortification and contribute to a reduction in fruit waste disposal problems.

2. Materials and Methods

2.1. Materials

A commercial air-dried sample of wild strawberry leaves (*F. vesca* L.) harvested in the summer of 2022 in the Dalmatia region, Croatia, was provided by the local specialized herbal drug store Suban Ltd. (Samobor, Croatia), and stored in a dark and dry place until analyzed. The sample belonged to batch line 22-045, with product lifetime ending in December 2023. The content of dry matter in the samples was determined to be 91.2 ± 0.2% by drying to constant mass [15].

2.2. Chemicals

All reagents used were analytical grade. Ultrapure water was obtained from a Milli-Q Plus water system (Millipore Corp., Bedford, NY, USA). Ethanol, anhydrous sodium carbonate (≥99.5%) was purchased from Lach-Ner (Neratovice, Czech Republic); methanol and Folin–Ciocalteu reagent were obtained from Merck (Darmstadt, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid, potassium persulfate, 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), and 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were from Sigma Aldrich (St. Louis, MO, USA). Hydrochloric acid (37%) and glacial acetic acid were provided by Carlo Erba Reagents (Val-de-Reuil, France). Iron (III)-chloride hexahydrate was supplied by Gram-mol d.o.o. (Zagreb, Croatia). Sodium acetate was purchased from Kemika d.d. (Zagreb, Croatia). Authentic standards of myricetin, caffeic acid, gallic acid, ferulic acid, protocatechuic acid, syringic acid, rosmarinic acid, chlorogenic and *p*-coumaric acid, quercetin-3-glucoside, quercetin-3-rutinoside, kaempferol-3-glucoside, kaempferol-3-rutinoside, catechin, epigallocatechin gallate, epicatechin gallate, apigenin, procyanidin B2, and luteolin were procured from Biovit d.o.o. (Jalkovec, Croatia).

2.3. Preparation of Phenolic Extracts

2.3.1. Microwave-Assisted Extraction (MAE)

The first series of extracts was obtained by MAE in an Ethos Easy reactor (Milestone, Sorisole, Italy). Appropriate sample masses corresponding to the SSRs (1.25, 1.67, 2.50 g) were transferred to the extraction cells with a magnetic stirrer and treated with 50 mL of an aqueous ethanol solution (30 vol. %). The cells were positioned on the rotor of a microwave reactor; the time required for temperature achievement was set at 4 min for 60 °C, 5 min for 70 °C, and 6 min for 80 °C at 800 W, and an automatic extraction process was started. Extraction parameters were set according to the experimental design listed in Section 3.3.1, with varying extraction SSRs (20, 30, and 40), extraction temperatures (60, 70, and 80 °C), and irradiation times (5 and 10 min), while the microwave power was constant throughout all trials (400 W). Afterward, the obtained extracts were filtered through Whatman No. 40 filter paper (Whatman International Ltd., Kent, UK) into volumetric 50 mL flasks and made up to volume with 30% ethanol, and the resulting supernatants were stored in the refrigerator until further analysis.

2.3.2. Accelerated Solvent Extraction (ASE)

The second series of extracts was obtained by ASE using a Dionex™ ASE™ 350 Accelerated Solvent Extractor (Thermo Fisher Scientific Inc., Sunnyvale, CA, USA). All extractions were performed in 34 mL stainless steel cells containing two cellulose filters (Dionex™ 350/150 Extraction Cell Filters, Thermo Fisher Scientific Inc., Waltham, MA, USA). Various sample masses corresponding to the SSRs (1.25, 1.67, 2.50 g) were transferred to the extraction cells with the addition of diatomaceous earth and an additional cellulose filter on the top. Ethanol (30 vol. %) was used as the extraction solvent. According to the parameters listed in Section 3.3.1, extractions were performed at three different SSRs (20, 30, and 40), three temperatures (100, 125, and 150 °C), and two different static extraction times (5 and 10 min). The extraction was performed by filling the cell containing the sample with the solvent up to a pressure of 10.34 MPa, after which the cell was rinsed with a rinse volume of 30%, followed by purging with N_2 gas for 30 s. Three extraction cycles were performed for every trial; extracts were collected in 250 mL glass vials, filtered through Whatman No. 40 filter paper (Whatman International Ltd., Kent, UK) into volumetric 50 mL flasks, and made up to volume with 30% ethanol. The resulting supernatants were stored in the refrigerator and further analyzed within 10 days.

2.4. Determination of Total Phenolic Content (TPC)

The Folin–Ciocalteu assay with some modifications [16] was carried out in order to determine the TPC of wild strawberry leaves. In brief, 100 µL of the extract (or 30% ethanol

for blank) was mixed with 200 µL of Folin–Ciocalteu reagent and 2 mL of distilled water. After 3 min, 1 mL of 20% sodium carbonate solution was added, and the mixture was vortexed. After 25 min incubation in a water bath at 50 °C, the absorbance was measured at 765 nm using a VWR UV-1600PC Spectrophotometer (VWR, Wayne, PA, USA). Gallic acid was used to prepare the standard curve for TPC, and the results are expressed as mg of gallic acid equivalent per 100 g of sample dry weight (mg GAE/100 g DW). The extract with the highest TPC for an individual extraction technique was filtered through 0.45 µm nylon syringe filters and further subjected to the phenolic profile analysis.

2.5. Determination of the Individual Phenolic Content

The identification and quantification of phenolics in wild strawberry leaf extracts obtained under optimized MAE and ASE conditions were performed in positive and negative ionization modes with an ESI ion source on an Agilent 6430 Triple Quad LC /MS mass spectrometer (Agilent, Santa Clara, CA, USA) connected to a UPLC system (Agilent Series 1290 RRLC instrument) and Agilent MassHunter Workstation software (Ver. B.04.01) for data processing and instrument control. N_2 was used as desolvation and collision gas with the following parameters: desolvation gas temperature 300 °C, flow rate 11 L/h, capillary voltage kV/−3.5 kV, and nebulizer pressure 40 psi. Agilent's Zorbax Eclipse Plus C18 column (100 × 2.1 mm; particle size 1.8 µm) was used for separations under the following conditions: column temperature 35 °C, injection volume 2.5 µm. The solvent composition and gradient conditions used were previously described [17]. The identification and quantitative determination were carried out on the basis of the calibration curves of the standards: myricetin, caffeic acid, gallic acid, ferulic acid, protocatechuic acid, syringic acid, rosmarinic acid, chlorogenic and *p*-coumaric acid, quercetin-3-glucoside, quercetin-3-rutinoside, kaempferol-3-glucoside, kaempferol-3-rutinoside, catechin, epigallocatechin gallate, epicatechin gallate, apigenin, procyanidin B2, and luteolin. For compounds lacking reference standards, identification was based on mass spectral data and literature reports of mass fragmentation patterns, while quantification was performed as follows: luteolin-6-C-glucoside and luteolin-7-*O*-rutinoside according to luteolin; apigenin pentoside and apigenin-6-C-(*O*-deoxyhexosyl)-hexoside according to apigenin; epicatechin according to catechin; procyanidin trimer and procyanidin B1 according to procyanidin B2; 3-p-caffeoylquinic acid, 3,5-di-caffeoylquinic acid, 4,5-di-caffeoylquinic acid, and 4-*O*-caffeoylquinic acid according to chlorogenic acid; 3-*O*-ferruylquinic acid according to ferulic acid; 5-*O*-galloylquinic acid, *p*-hydroxybenzoic acid, and 3,5-di-galloylquinic acid according to gallic acid. Quercetin-3-glucuronide, quercetin-3-rhamnoside, quercetin-3-pentoside, quercetin-acetyl-hexoside, isorhamnetin-3-*O*-glucoside, quercetin-acetyl-rutinoside, quercetin-3-*O*-dihexoside, isorhamnetin-pentosylhexoside, quercetin-3-*O*-vicianoside, quercetin, and quercetin-pentosylhexoside were identified according to quercetin-3-glucoside; kaempferol-3-*O*-hexoside, kaempferol-3-glucuronide, kaempferol-3-*O*-deoxyhexoside, kaempferol-3-*O*-pentoside, kaempferol-pentosyl-hexoside, kaempferol-acetyl-hexoside, and kaempferol according to kaempferol-3-*O*-glucoside; myricetin-3-*O*-rhamnoside and myricetin-3-*O*-galactoside according to myricetin; kaempferol-acetyl-rutinoside according to kaempferol-3-rutinoside. Quality parameters, including instrumental detection (LOD) and quantification (LOQ) limits, as well as calibration curves, were reported previously [17]. The concentrations of the analyzed phenolics are expressed as mg per 100 g of dry leaf and reported as the mean value ± standard deviation.

2.6. Determination of Antioxidant Properties

2.6.1. ABTS

The radical-scavenging activity of wild strawberry leaf extracts against the $ABTS^{\bullet+}$ radical was carried out as described previously [18] with some modifications. In brief, a stable ABTS radical cation ($ABTS^{\bullet+}$) solution was prepared by reacting the ABTS stock solution (7 mM) with a potassium persulfate solution (140 mM) as the oxidizing agent. The next day, the $ABTS^{\bullet+}$ water solution was diluted with ethanol until reaching an absorbance

value of (0.700 ± 0.020) at 734 nm. The appropriately diluted extract (160 µL) was mixed with the $ABTS^{\bullet+}$ solution (2 mL), the reaction mixture was vortexed, and after 1 min incubation, the absorbance was measured at 734 nm. Ethanol (96 vol. %) was used as a blank. Trolox was used as the standard to establish a standard curve, and the results are expressed as µmol of Trolox equivalent per 1 g of sample dry weight (µmol TE/g DW).

2.6.2. DPPH

The radical-scavenging activity of wild strawberry leaf extracts against the $DPPH^{\bullet}$ radical was conducted as described previously [19] with some modifications. In total, 0.75 mL of the extract was mixed with 1.5 mL of freshly prepared 0.2 mM $DPPH^{\bullet}$ solution in methanol, and the mixture was stored in the dark at room temperature for 20 min. Then, the absorbance of the mixture was measured at 517 nm against methanol as a blank. Trolox was used as the standard, and results are given as Trolox equivalent (µmol TE/g DW).

2.6.3. Ferric Reducing Antioxidant Power (FRAP)

The reducing properties of wild strawberry leaf extracts were further evaluated by the FRAP assay with some modifications [16]. The FRAP reagent was produced using a sodium acetate buffer (0.3 M, pH 3.6), TPTZ (0.01 M solution in 0.04 M hydrochloric acid), and $FeCl_3 \times 6H_2O$ aqueous solution (20 mM) in a ratio of 10:1:1. In total, 240 µL of distilled water was mixed with 80 µL of the extract (extraction solvent for blank) and 2080 µL of freshly prepared FRAP reagent. The reaction mixture was incubated for 5 min at 37 °C, and absorbance was measured at 593 nm. Trolox was used as the standard, and results are given as Trolox equivalent (µmol TE/g DW).

2.7. Statistical Analysis

For the evaluation of the effect of MAE and ASE parameters on the TPC of wild strawberry leaf extracts, a mixed 2- and 3-level full factorial design was employed, where the SSR and temperature were factors observed at 3 levels, while the extraction time was observed at 2 levels. The extractions were performed in two parallel runs, and all of the analyses were performed in duplicate. Statistical analysis was conducted by using SPSS for Windows (Version 21). Data are presented as means with standard deviations. The values of the tested dependent variables (TPC, ABTS, FRAP, DPPH) were tested for homoscedasticity (Levene's test) and normality (Shapiro–Wilk test). Normal and homoscedastic data sets were analyzed by a one-way analysis of variance (ANOVA), while the samples that did not meet those requirements were analyzed by a non-parametric Kruskal–Wallis test with manual post hoc to identify significant differences (for all tests, $p \leq 0.05$). Pearson's correlation coefficients were used to establish the relationship between the studied responses.

3. Results and Discussion

Extraction represents the separation and recovery of the desired compounds from the plant matrix without compromising their functionalities [13]. As far as we know, this is the first report of the green extraction of phenolics from wild strawberry leaves using MAE and ASE. As for the variables investigated in the present study, the qualitative and quantitative properties of the extracts obtained can be strongly influenced by the extraction technique and the predetermined factors, such as the temperature, SSR, and extraction time.

3.1. Effects of Extraction Parameters on Yield of Phenolics

The TPC results of the two green extraction techniques are shown in Figure 1 (MAE) and Figure 2 (ASE). The yield of the extracted phenolics from wild strawberry leaves ranged from 3446 to 6313 mg GA/100 g DW for MAE, while the yield obtained with ASE was higher, ranging from 5123 to 8027 mg GA/100 g DW.

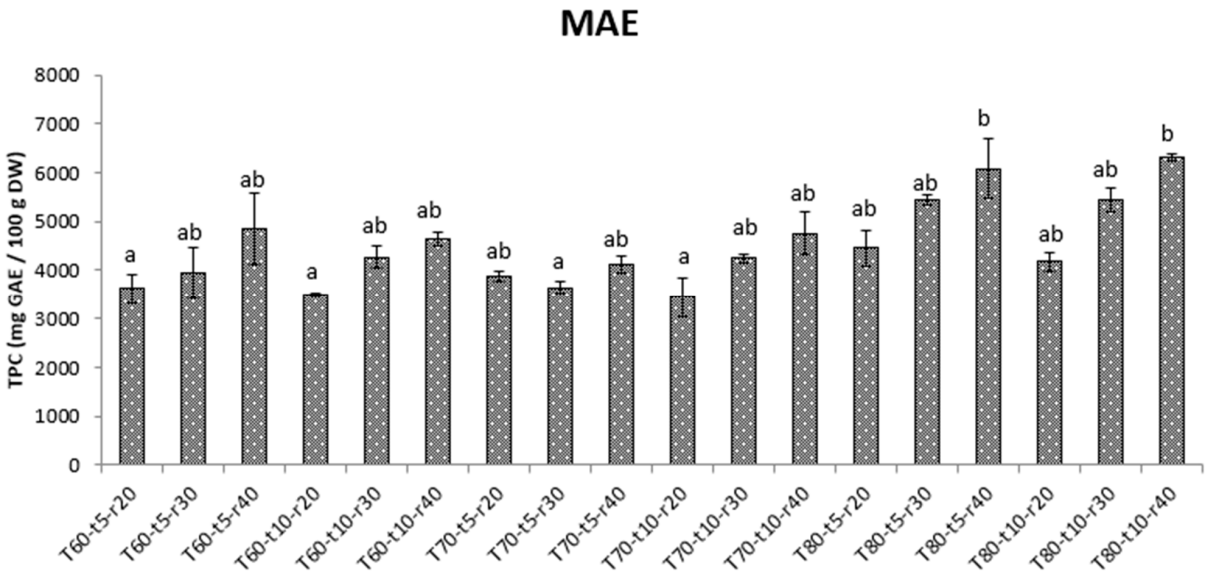

Figure 1. Total phenolic content (TPC) of wild strawberry leaves obtained by microwave-assisted extraction (MAE) using different combinations of extraction parameters: temperatures of 60, 70, and 80 °C (T60, T70, T80), extraction times of 5 and 10 min (t5, t10), and solvent-to-sample ratios of 20, 30, and 40 mL/g (r20, r30, r40). Data are expressed as mean ± standard deviation. Different letters indicate statistically significant differences according to Kruskal–Wallis test ($p < 0.05$).

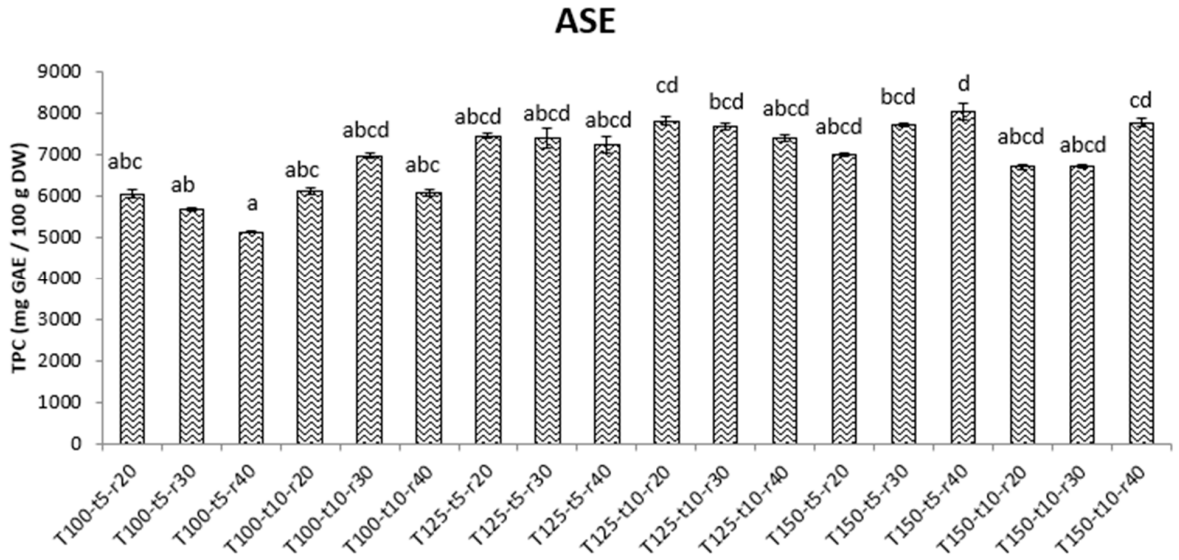

Figure 2. Total phenolic content (TPC) of wild strawberry leaves obtained by accelerated solvent extraction (ASE) at different combinations of extraction parameters: temperatures of 100, 125, and 150 °C (T100, T125, T150), static extraction times of 5 and 10 min (t5, t10), and solvent-to-sample ratios of 20, 30, and 40 mL/g (r20, r30, r40). Data are expressed as mean ± standard deviation. Different letters indicate statistically significant differences according to Kruskal–Wallis test ($p < 0.05$).

In the system of MAE, the rupture of cell walls is caused by the extreme internal pressure generated by the rapid evaporation of the constitutive water impacted by electromagnetic rays [13]. As can be seen in Figure 1, the extraction temperature had a moderate influence on this process. The mutual comparison of the treatments, where the temperature was varied and the time and SSR were kept constant, showed that a similar content of TPC was obtained with extraction at 60 and 70 °C. In contrast, the highest extraction temperature (80 °C) resulted in an almost one-third higher yield of TPC compared to 60 °C. As previously mentioned [13], a high temperature leads to the high solubility of the target

compounds in the extraction solvent, reduces the viscosity of the extraction solvent, and therefore allows inter- and intramolecular compounds to easily penetrate the disrupted cell walls. However, our results are not in accordance with a recent study [20] performed on *Phyllanthus niruri* leaves (MAE: 500 W, 2 min, 12:1 mL/g, varying temperatures from 40 to 70 °C), where the maximum value of phenolics was obtained at a temperature of 50 °C. Since these leaves have a different phenolic profile from wild strawberry leaves, it is possible that in the above-mentioned study, the degradation of sensitive compounds occurred as a result of higher temperatures [21].

The results of this study indicate that the SSR is another important parameter affecting the yield of phenolics obtained by the MAE technique, with 40:1 (hereinafter expressed in mL/g) giving the highest extraction yields of TPC (Figure 1). In contrast to conventional extraction techniques, where more solvent is usually associated with better extraction efficiency, more solvent in MAE can reduce the extraction efficiency. If the ratio is too high, the microwaves may be mainly absorbed by the main solvent, and a sufficient number of microwaves cannot reach the sample to cause the internal heating of the matrix, which may prevent the occurrence of cell disruption [22]. In addition, larger amounts require higher microwave energies, which greatly increases the heating of the solvent and/or sample and consequently increases the risk of the thermal degradation of the target molecules [14]. Moreover, it can be seen (Figure 1) that trials with the same SSR did not differ significantly from each other, regardless of the extraction temperature and time applied. These results contradict some previous findings under the given conditions (MAE: 500 W, 40 °C, 2 min, varying SSRs from 10:1 to 16:1), where a ratio of 12:1 provided the highest TPC yields from *P. niruri* leaves [20]. As stated by the authors, the reduction in yield observed beyond 12:1 could be due to the higher volume of solvents, which tend to require more time to reach equilibrium. Similar results were obtained in another study [23] in which, among the studied factors (almond skin weight, microwave power (100, 200, and 300 W), and irradiation time (20, 40, and 60 s)), the SSR had the greatest influence on the TPC response, showing a positive effect (SSR 17:1 > 30:1 >> 120:1). Our observation is more consistent with research on passion fruit peels (MAE: 240 W, 2 min), where 30:1 was found to be optimal among the tested ratios (10:1–50:1) [24]. Moreover, a study on strawberry (*Fragaria* × *ananassa* D.) leaves (MAE: 400 W, 40 s) showed that TPC increased significantly with the increase in the SSR (20:1–70:1), reaching a maximum at 60 mL/g [25]. However, a direct comparison with the literature is difficult due to the differences in materials, target molecules, operating parameters, and extraction conditions.

Figure 1 also presents the influence of the extraction time on the recovery of phenolics from wild strawberry leaves. In general, holding the other two parameters constant, the extraction solvent efficiently absorbed the microwave energy and resulted in the increased swelling of the leaves after only 5 min, whereas there was no statistically significant improvement in the TPC yield when the samples were extracted for 10 min instead of 5 min. However, the results obtained in this study indicate that at the optimal SSR (40:1), the longer extraction resulted in a 5% reduction, a 16% increase, and a 4% increase in TPC yield at 60, 70, and 80 °C, respectively. Similar results were also reported in the literature with *P. niruri* leaves (MAE: 200 W, 40 °C, 10:1, varying extraction times from 1 to 6 min). As indicated by the authors [20], the MAE took place in two phases: the first phase was the washing phase, and the second was the diffusion phase. The washing of phenolics from the *P. niruri* leaves (from 0 to 2 min) increased rapidly and reached the maximum yield at 2 min when the diffusion phase started, as the recovery of phenolics from the *P. niruri* leaves slowly increased. The latter results are consistent with another study performed on passion fruit peels (MAE: 400 W, 30:1, varying extraction times from 1 to 5 min), where the TPC increased when the microwave irradiation time was changed from 1 to 2 min and decreased when the time increased from 3 to 4 min [24]. An even shorter extraction time (10–60 s) was tested on strawberry leaves (*Fragaria* × *ananassa* D.) (MAE: 300 W, 50:1); the TPC reached its maximum value after 40 s [25]. However, the extraction time is closely related to the dielectric properties of the extraction solvent. Generally, the amount of an analyte increases

with increasing extraction time. Nevertheless, extracts of lower quality and with lower yields are possible due to the disruption of the structural integrity of chemically active phenolics within plant matrices [13]. In the present study, 10 min was a relatively long extraction time for MAE, and it should be considered that a reduction in the extraction time protects the matrices from enzymatic degradation [14].

Furthermore, the results of the present study showed that ASE is a more efficient technique for obtaining higher TPC yields from wild strawberry leaves. The accelerated diffusion of the analytes from the inner to the outer surface of the solid matrix is enabled by the breaking of intermolecular forces (Van der Waals forces, dipole–dipole interactions, hydrogen bonding) between the analytes and the active sites of the matrices as a result of the application of high temperatures. The increased pressure keeps the solvent in the liquid state at high temperatures, forces the solvent to flow through the solid matrix, even into the small pores and through the filter wherever possible, and ensures the continuous flow of the solvent, which favors mass transfer [13]. According to the results shown in Figure 2, extraction temperatures of 125 and 150 °C resulted in a higher extraction efficiency compared to 100 °C. The higher TPC in the extracts obtained at 125 and 150 °C could be partly related to the formation of Maillard reaction products (such as melanoidins), which can reduce the Folin–Ciocalteu reagent [26]. Similar results were also reported for the TPC yield from chaga [27], where the TPC yield gradually increased with increasing extraction temperature (40–200 °C) and reached a maximum at 200 °C. In agreement with the results of the above study, it seems that the extraction temperature significantly affects the phenolic recovery in ASE.

Due to the non-specificity of the Folin–Ciocalteu reagent, it is possible that non-phenolic reducing interferents (some sugars, organic acids, amino acids) lead to inaccurate estimates of TPC values. For example, in strawberry, which is known for its high flavonoid and vitamin C contents, the elimination of interferents significantly reduced the obtained TPC values [28]. However, our study was performed on leaves, not fruit. On the other hand, compounds formed by caramelization [29] and the Maillard reaction [26] may not have a phenolic structure but may have antioxidant properties. In addition, thermal decarboxylation of hydroxycinnamic acids to the corresponding 4-vinyl derivatives should also be considered, since decarboxylation reduces the reduction and antiradical activity of the corresponding phenolic acids in a homogeneous polar medium [30].

Our experimental data are in line with a similar study [31] performed on the brown seaweed *Fucus vesiculosus*, in which an extraction temperature of 140 °C resulted in a 6-fold higher TPC compared to 110 °C, while the results obtained with the DPPH and ABTS methods did not follow this trend. However, others [32] have also pointed out that the major shortcomings of ASE are the low analyte selectivity during extraction and the presence of interferents during the extraction process.

Figure 2 shows the influence of the static extraction time on the recovery of wild strawberry phenolics obtained by ASE. Although no statistically significant differences were observed between treatments with variable times and a constant temperature and SSR, some important changes occurred. Interestingly, prolonged extraction at 100 °C and an SSR of 40:1 resulted in an 18.4% higher TPC yield, while at 150 °C and an SSR of 30:1, the effect of the extraction time was negative (13% decrease). The effect of the extraction time was also investigated in previous research [27], in which chaga phenolics were analyzed using six static periods (1, 5, 7, 10, 15, and 20 min). The maximum TPC was reached after 7 and 10 min. While 5 min was not yet sufficient for a complete extraction at 130 °C, an extraction time of 15 min led to a decrease in TPC, probably due to thermal degradation.

In the present study, the effect of the SSR on the ASE efficiency of TPC was investigated by extracting different amounts of wild strawberry leaves (1.25, 1.67, 2.50 g) with the same amount of extraction solvent. All extractions were performed in stainless steel cells of the same size, and the final volume was adjusted to 50 mL. As shown in Figure 2, the increase in TPC was not proportional to the sample quantity, regardless of the extraction temperature or static extraction time. Moreover, opposite results were obtained when 5 min

extraction was performed at 100 °C, where a lower sample mass resulted in a 15% lower TPC yield, and at 150 °C, where a lower sample mass resulted in a 15% higher TPC yield (comparison between SSRs of 40:1 and 20:1). Based on previous reports [33], the reduction in extraction efficiency may be due to poor interaction between the solid and the solvent, possibly due to the caking of the sample reducing the solubility of the phenolics in the extraction solvent. In the aforementioned study, performed on potato peel, the optimal SSR was found to be 80:1, with SSRs down to 16:1 being tested.

In summary, the highest TPC among all 36 experiments tested was obtained with 5 min ASE at 150 °C and an SSR of 40:1 (8027 ± 194 mg GAE/100 g DW).

3.2. Effects of Extraction Methods on Phenolic Profile

UPLC/MS-MS analysis was performed to investigate the phenolic profile of wild strawberry leaf extracts obtained using the defined optimal MAE and ASE parameters. A total of 54 phenolics, consisting of phenolic acids, proanthocyanidins, flavonols, flavan-3-ols, and flavones, were identified through a comparison with their authentic standards or on the basis of their molecular formulas, exact mass measurements, and MS/MS fragmentations, as presented in previous works [34–37] by our experts (specified below). Among the phenolic acids, compounds 1, 3, 5, 12, 13, and 39 were identified as ferulic, rosmarinic, chlorogenic, syringic, caffeic, and gallic acids, respectively, through a comparison with authentic standards. To the best of our knowledge, this is the first report on the detailed profile of phenolic acids in wild strawberry leaves. Compounds 2, 8, 9, 17, 25, 28, and 53 were identified according to previously described [34] fragmentation patterns as 3-*p*-caffeoylquinic acid, 3,5-di-caffeoylquinic acid, 4,5-di-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 5-*O*-galloylquinic acid, 3-*O*-ferruylquinic acid, and 3,5-digalloylquinic acid, respectively. The presence of quinic acid and 4-*O*-caffeoylquinic acid in wild strawberry leaves is consistent with previous studies [10,11], while other derivatives were identified for the first time. Compound 40 was identified as *p*-hydroxybenzoic acid according to recently published data [35]. The presence of *p*-hydroxybenzoic acid in wild strawberry leaves is in accordance with the literature [11]. Among the proanthocyanidins, compound 10 was identified as procyanidin B2 through a comparison with the authentic standard. Compound 4 was identified according to a recently published [35] fragmentation pattern as a procyanidin trimer, while compound 30 was identified as procyanidin B1 [34]. To the best of our knowledge, this is the first study to report the presence of a procyanidin trimer. Among flavonols, compounds 15, 35, 44, and 47 were identified through a comparison with authentic standards as myricetin, quercetin-3-glucoside, rutin, and kaempferol-3-rutinoside, respectively. Compounds 26, 34, and 37 were identified according to the published data [36] as myricetin-3-*O*-rhamnoside, myricetin-3-*O*-galactoside, and myricetin-3-*O*-arabinoside, respectively. To the best of our knowledge, our study is the first to identify derivatives of myricetin in the leaves of wild strawberry. Compounds 16 and 18 were identified as quercetin-3-glucuronide and kaempferol-3-glucuronide, respectively, according to previous work [37]. Compounds 6, 7, 11, 21, and 27 were identified as isorhamnetin-3-rhamnoside, isorhamnetin-3-hexoside, kaempferol-3-*O*-hexoside, kaempferol-3-*O*-deoxyhexoside, and kaempferol-3-*O*-pentoside, respectively, following previous work [35]. As far as we can tell, our study is the first to identify derivatives of isorhamnetin in the leaves of wild strawberry. Compounds 19, 23, 29, 31, 32, 41, 42, and 51 were identified according to recent findings [36] as quercetin-3-rhamnoside, quercetin-3-pentoside, kaempferol-pentosyl-hexoside, quercetin-acetyl-hexoside, kaempferol-acetyl-hexoside, quercetin-acetyl-rutinoside, kaempferol-acetyl-rutinoside, and quercetin-pentosylhexoside, respectively. Compounds 33, 43, 45, 49, and 50 were identified according to the published data [17] as isorhamnetin-3-*O*-glucoside, quercetin-3-*O*-dihexoside, isorhamnetin-pentosylhexoside, quercetin, and kaempferol, respectively. Compound 46 was identified as quercetin-3-vicianoside, as reported before [38]. The presence of quercetin, kaempferol, rutin, and some of their derivatives in wild strawberry leaves has been previously confirmed [4,11], but our paper is the first detailed research on various flavonols derivatives, as we characterized

as many as eight kaempferol and nine quercetin derivatives. Among the flavan-3-ols, compounds 36 and 54 were identified through a comparison with authentic standards as epigallocatechin gallate and epicatechin gallate, respectively. Compound 24 was identified as epicatehin, as previously covered [35]. The presence of epicatechin and epicatechin gallate in wild strawberry leaves is consistent with the literature [4,11]. Among the flavones, compounds 20 and 22 were identified as luteolin and apigenin through comparison with authentic standards. Compounds 14 and 48 were identified according to recent work [35] as luteolin-6-*C*-glucoside and apigenin-6-*C*-(*O*-deoxyhexosyl)-hexoside, respectively. Compounds 38 and 52 were identified as apigenin pentoside and luteolin-7-*O*-rutinoside according to a previously described [17] fragmentation pattern. Based on our information, this study is the first to report the detection of various flavones and their derivatives in wild strawberry leaves.

As recently summarized [14], MAE is most useful for short-chain phenolics (e.g., phenolic acids, flavonoids), which are stable with microwave heating up to 100 °C. In this study, a 40% higher content of total phenolic acids was obtained with the MAE technique at 80 °C than with ASE at 150 °C (Figure 3). Among the phenolic acids identified (Table 1), the same four compounds were the most abundant in both extracts (ranging between 172 and 40 mg/100 g) but in different descending orders (MAE: *p*-hydroxybenzoic acid > gallic acid >> 5-*O*-galloylquinic acid > chlorogenic acid; ASE: *p*-hydroxybenzoic acid >> 5-*O*-galloylquinic acid > chlorogenic acid ≈ gallic acid). According to the literature, the number and type of substituents, as well as the position of the hydroxyl group, influence the phenolics' thermal stability, but the latter was only partially confirmed in this study, where similar contents of chlorogenic (5-*O*-caffeoylquinic acid), 3,5-di-caffeoylquinic acid, and 4,5-di-caffeoylquinic acid were obtained with both extraction techniques, with MAE proving to be a significantly (by 109%) better option for the extraction of 4-*O*-caffeoylquinic acid. Moreover, the yield of caffeic acid was more affected by the extraction technique (22% increase with MAE) than that of its esterified form with quinic acid (chlorogenic acid, 9% increase) and that of its dimer (rosmarinic acid, 0%). Interestingly, the results of this study showed better extraction efficiency for *p*-hydroxybenzoic acid (possessing one hydroxyl group) and gallic acid (with three hydroxyl groups) with MAE at 80 °C than with ASE at 150 °C, with increases of 22% and as high as 294%, respectively, indicating that phenolic acids with more hydroxyl groups are less stable at high temperatures. In the present case, ASE resulted in a 20% higher yield of 3,5-digalloylquinic acid, but this trend could not be confirmed for the other quinic acid esters identified in this study. In addition, it has been previously reported [14] that hydroxylates are more prone to chemical alteration during MAE than methoxylates, which is in line with our results for ferulic acid (having one methoxy group) and syringic acid (having two methoxy groups), whose ASE resulted in 16 and 62% higher yields than MAE, respectively. Furthermore, UPLC/MS-MS analysis of the ethanolic extracts identified three proanthocyanidins (Table 1), and the results revealed that under optimal extraction conditions, a total proanthocyanidin content of 397.8 mg/100 g was obtained using ASE, whereas only 47.5 mg/100 g was obtained with MAE (Figure 3). Procyanidin B1 represented 94% of total proanthocyanidins in the MAE extract and 84% in the ASE extract and was also found to be the major proanthocyanidin in the aqueous extract of wild strawberry leaves, as determined by others [4]. In our study, the presence of several flavonols was confirmed in both extracts (Table 1), including quercetin, kaempferol (over 250 mg/100 g), quercetin-3-glucuronide (over 125 mg/100 g), myricetin-3-*O*-galactoside (above 50 mg/100 g), rutin, myricetin, kaempferol-3-glucuronide (above 30 mg/100 g), and quercetin-3-glucoside (above 10 mg/100 g) in greater quantities, regardless of the extraction technique. In line with the present results for wild strawberry, quercetin and kaempferol and their derivatives were the predominant flavonol groups in strawberry (*Fragaria* × *ananassa*) leaves [39,40] and were also the major constituents of low-molecular-weight phenolic compounds in the leaves of black currant and raspberry [39]. The extraction yield of the following flavonols was strongly influenced by the type of extraction: for quercetin-3-pentoside, myricetin-3-*O*-rhamnoside, quercetin-acetyl-rutinoside,

and kaempferol-acetyl-rutinoside, ASE was the significantly more efficient technique, while kaempferol-3-*O*-pentoside and kaempferol-acetyl-hexoside were considerably better extracted with MAE (Table 1). Overall, a 29% higher content of flavonols was obtained with ASE at 150 °C than with MAE at 80 °C (Figure 3). Our observations are not fully consistent with the research on *Moringa oleifera* leaves [41], which suggested that MAE (158 °C) enables better recovery of kaempferol, quercetin, and their glucoside derivatives when compared to ASE (128 °C). However, other glycosylated flavonoids with a higher number of hydroxyl-type substituents were better extracted from *M. oleifera* under ASE conditions. Although the extracts obtained under MAE and ASE conditions had similar qualitative compositions in the aforementioned cited study, the authors concluded that the extraction method should be selected depending on the target molecules, as not all derivatives of flavonoids followed the same trend, which is consistent with our results.

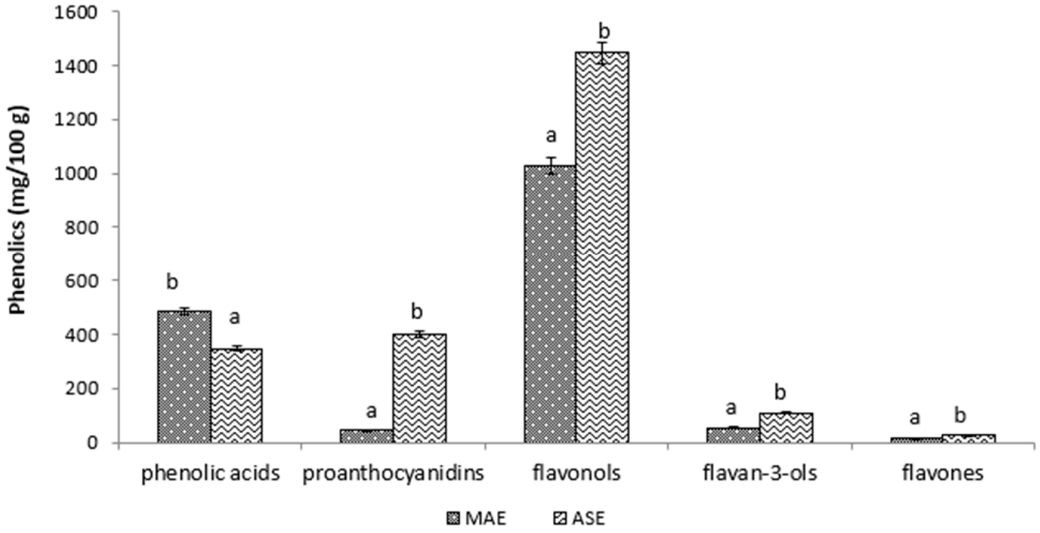

Figure 3. Contents of different classes of phenolics as determined by UPLC/MS-MS in extracts of wild strawberry leaves obtained by microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE) under optimal conditions. Data are expressed as mean ± standard deviation. Different letters indicate statistically significant differences ($p < 0.05$).

Table 1. Contents of individual phenolic acids, proanthocyanidins, flavonols, flavan-3-ols, and flavones determined in extracts of wild strawberry leaves obtained by microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE) under optimal conditions. Data are expressed as mean ± standard deviation. Different letters indicate statistically significant differences ($p < 0.05$).

No.	Compound Name	Retention Time (min)	*m/z*	*m/z* (Prod.)	MAE mg/100 g	ASE mg/100 g
			phenolic acids			
1	ferulic acid *	1.937	193	178	5.13 ± 015 ^{a}	6.07 ± 0.17 ^{b}
2	3-*p*-caffeoylquinic acid	2.906	337	163	3.64 ± 0.10 ^{b}	2.90 ± 0.08 ^{a}
3	rosmarinic acid *	3.138	359.1	161	16.74 ± 0.47 ^{a}	16.82± 0.48 ^{a}
5	chlorogenic acid *	4.615	353	191	43.57 ± 1.23 ^{a}	40.06 ± 1.13 ^{a}
8	3,5-di-caffeoylquinic acid	5.573	515	173	0.83 ± 0.02 ^{b}	0.76 ± 0.02 ^{a}
9	4,5-di-caffeoylquinic acid	5.573	515	353	1.12 ± 0.03 ^{a}	1.13± 0.03 ^{a}
12	syringic acid *	6.354	197	182	7.39 ± 0.21 ^{a}	19.42± 0.55 ^{b}
13	caffeic acid *	6.368	179	135	8.44 ± 0.24 ^{b}	6.91± 0.20 ^{a}
17	4-*O*-caffeoylquinic acid	7.821	324	173	0.15 ± 0.00 ^{b}	0.07± 0.00 ^{a}
25	5-*O*-galloylquinic acid	9.775	343	191	61.38 ± 1.74 ^{a}	62.22± 1.76 ^{a}
28	3-*O*-ferruylquinic acid	11.238	367	193	1.77 ± 0.05 ^{a}	1.42± 0.04 ^{a}
39	gallic acid *	11.528	169	125	156.96 ± 4.44 ^{b}	39.84± 1.13 ^{a}
40	*p*-hydroxybenzoic acid	11.538	137	93	172.41 ± 4.88 ^{b}	141.78 ± 4.01 ^{a}
53	3,5-Digalloylquinic acid	11.968	495	343	5.19 ± 0.15 ^{a}	6.50 ± 0.18 ^{b}

Table 1. *Cont.*

No.	Compound Name	Retention Time (min)	*m/z*	*m/z* (Prod.)	MAE mg/100 g	ASE mg/100 g
			proanthocyanidins			
4	procyanidin trimer	3.438	865	575	1.84 ± 0.05 [a]	43.71± 1.24 [b]
10	procyanidin B2 *	5.815	577	289	0.92 ± 0.03 [a]	21.84± 0.62 [b]
30	procyanidin B1	11.351	579	291	44.73 ± 1.27 [a]	332.26 ± 9.40 [b]
			flavonols			
6	isorhamnetin-3-rhamnoside	5.178	625	317	1.13 ± 0.03 [a]	1.72 ± 0.05 [b]
7	isorhamnetin-3-hexoside	5.232	479	317	7.84 ± 0.22 [b]	2.06 ± 0.06 [a]
11	kaempferol-3-*O*-hexoside	6.252	449	287	5.36 ± 0.15 [a]	5.69 ± 0.16 [a]
15	myricetin *	7.258	319	273	48.33 ± 1.37 [b]	33.19 ± 0.94 [a]
16	quercetin-3-glucuronide	7.442	479	303	129.31 ± 3.66 [a]	268.97 ± 7.61 [b]
18	kaempferol-3-glucuronide	8.192	463	287	39.79 ± 1.13 [a]	70.47 ± 1.99 [b]
19	quercetin-3-rhamnoside	8.213	449	303	2.30 ± 0.06 [a]	3.97 ± 0.11 [b]
21	kaempferol-3-*O*-deoxyhexoside	8.475	433	286	1.40 ± 0.04 [a]	1.93 ± 0.05 [a]
23	quercetin-3-pentoside	9.700	435	303	1.68 ± 0.05 [a]	11.81 ± 0.33 [b]
26	myricetin-3-*O*-rhamnoside	9.905	465	319	6.13 ± 0.17 [a]	43.71 ± 1.24 [b]
27	kaempferol-3-*O*-pentoside	10.689	419	287	3.08 ± 0.09 [b]	0.91 ± 0.03 [a]
29	kaempferol-pentosyl-hexoside	11.344	581	287	0.25 ± 0.01 [a]	0.77 ± 0.02 [b]
31	quercetin-acetyl-hexoside	11.357	507	303	1.41 ± 0.04 [a]	2.55 ± 0.07 [b]
32	kaempferol-acetyl-hexoside	11.361	491	287	1.22 ± 0.03 [b]	0.34 ± 0.01 [a]
33	isorhamnetin-3-*O*-glucoside	11.364	483	317	1.41 ± 0.04 [b]	0.83 ± 0.02 [a]
34	myricetin-3-*O*-galactoside	11.368	481	319	68.99 ± 1.95 [b]	52.33 ± 1.48 [a]
35	quercetin-3-glucoside *	11.381	465	303.1	11.45 ± 0.32 [a]	37.48 ± 1.06 [b]
37	myricetin-3-*O*-arabinoside	11.395	451	319	8.37 ± 0.24 [b]	4.36 ± 0.12 [a]
41	quercetin-acetyl-rutinoside	11.552	653	303	0.28 ± 0.01 [a]	1.29 ± 0.04 [b]
42	kaempferol-acetyl-rutinoside	11.556	637	287	0.12 ± 0.00 [a]	0.54 ± 0.01 [b]
43	quercetin-3-*O*-dihexoside	11.559	627	303	2.74 ± 0.08 [b]	1.97 ± 0.06 [a]
44	rutin *	11.566	611	303	44.31 ± 1.25 [a]	116.04 ± 3.28 [b]
45	isorhamnetin-pentosylhexoside	11.566	611	317	1.23 ± 0.03 [b]	0.43 ± 0.01 [a]
46	quercetin-3-*O*-vicianoside	11.576	597	434	2.77 ± 0.08 [b]	1.82 ± 0.05 [a]
47	kaempferol-3-rutinoside *	11.586	595	287	3.56 ± 0.10 [a]	7.54 ± 0.21 [b]
49	quercetin	11.681	303	303	336.35 ± 9.51 [a]	472.63 ± 13.34 [b]
50	kaempferol	11.698	287	287	296.57 ± 8.39 [a]	298.96 ± 8.46 [a]
51	quercetin-pentosylhexoside	11.825	597	303	1.42 ± 0.04 [a]	2.67 ± 0.08 [b]
			flavan-3-ols			
24	epicatechin	9.727	291	139	45.42 ± 1.28 [a]	100.29 ± 2.84 [b]
36	epigallocatechin gallate *	11.388	459	289	5.41 ± 0.15 [a]	5.48 ± 0.15 [a]
54	epicatechin gallate *	12.149	442.9	273	3.53 ± 0.10 [b]	2.26 ± 0.06 [a]
			flavones			
14	luteolin-6-*C*-glucoside	6.978	449	359	2.73 ± 0.08 [b]	0.80 ± 0.02 [a]
20	luteolin *	8.264	287	153	9.82 ± 0.28 [a]	17.75 ± 0.50 [b]
22	apigenin *	8.758	271	153	0.34 ± 0.01 [a]	0.56 ± 0.02 [b]
38	apigenin pentoside	11.429	403	271	0.399 ± 0.01 [a]	0.55 ± 0.02 [b]
48	apigenin-6-*C*-(*O*-deoxyhexosyl)-hexoside	11.593	579	459	0.24 ± 0.01 [a]	0.57 ± 0.02 [b]
52	luteolin-7-*O*-rutinoside	11.828	595	287	3.45 ± 0.109 [a]	7.51 ± 0.21 [b]
	total phenolics				1632.32 ± 26.17 [a]	2326.42 ± 65.80 [b]

* Identification confirmed with authentic standards. Values within rows marked with different letters are significantly different at $p < 0.05$.

In addition to a high antioxidant potential [10], comparable to the antioxidant capacity of white wines [8], the compounds extracted from wild strawberry leaves are able to inhibit nitrite production, which is achieved by a direct nitric oxide-scavenging effect [2], and can protect against cardiovascular diseases, probably due to the synergistic action of different

phenolics [4]. According to the literature, condensed tannins and flavonoid derivatives seem to be responsible for the antioxidant activity of *F. vesca* leaves [11]. On the other hand, another study found only a moderate correlation between total proanthocyanidins in the investigated extracts and their antioxidant activity [9]. However, the newly identified compounds in the present study, namely, chlorogenic acid, gallic acid, rosmarinic acid, ferulic acid, and caffeic acid, are well known for their antioxidant, antitumor, and antimicrobial properties, among others [42]. For example, gallic acid showed antitumor activity in prostate and brain carcinoma cells, syringic acid expressed antibacterial and hepatoprotective activity, caffeic and ferulic acids showed antioxidant activity and antimicrobial activity against pathogenic bacteria and fungi, and chlorogenic acid was able to significantly reduce the invasion of a rat ascites hepatoma cell line without altering cell proliferation [42]. Moreover, myricetin has antiviral potential, suppresses pathogenic microbial infections, and has a health-promoting effect on various tumors, inflammatory diseases, atherosclerosis, thrombosis, cerebral ischemia, diabetes, Alzheimer's disease, etc. [43]. In addition, isorhamnetin and its derivatives are associated with cardiovascular and cerebrovascular protection, antitumor, anti-inflammatory, antioxidation effects, organ protection, the prevention of obesity, etc. [44]. The biological activities of luteolin include antioxidant, antibacterial, anti-inflammatory, and anti-cancer activities [45], while apigenin and its derivatives have therapeutic effects on cancer, diabetes, obesity, depression, insomnia, infections, and respiratory, cardiovascular, hepatoprotective, neurodegenerative, and skin diseases [46].

The literature [14] states that more complex phenolics with numerous hydroxyl conjugates are unsuitable as MAE targets, since they can be structurally damaged by microwave energy. Flavan-3-ols were also detected in both of our extracts, among which epicatechin was more prevalent, with 84 and 93% in the MAE and ASE extracts, respectively, but it should be emphasized that a much higher absolute content of epicatechin (100.3 mg/100 g) was obtained with ASE than with MAE under optimal conditions (45.4 mg/100 g). Epicatechin was also quantified in significant amounts in the aqueous extract of wild strawberry leaves [4], where, in contrast to our results, epigallocatechin was more prevalent. In addition, ASE resulted in a 39% higher yield of flavones compared to MAE (Figure 3). Luteolin and its derivatives represented 94% of the total flavones (Table 1), regardless of the extraction technique, with their absolute contents being 16.0 and 26.1 mg/100 g in the MAE and ASE extracts, respectively. The type of extraction also affected apigenin and its derivatives, with significantly higher yields obtained with ASE. ASE appears to be a more efficient method for the extraction of flavones than conventional and ultrasound-assisted extraction, as the yield of flavones in the leaves of bay, sage, thyme, and myrtle was greatly improved by ASE, as published recently [47].

In this study, the amounts of each individual compound were found to be about 10-fold lower than in the previous work [25], in which the following phenolics (in descending order) were identified in the extracts of strawberry leaves (*Fragaria* × *ananassa* D.) obtained by MAE (300 W, 40 s, 61.6 mL/g): sinapic acid (55.74 ± 2.45 mg/g), rutin (8.08 ± 0.87 mg/g), epicatechin (5.35 ± 0.94 mg/g), catechins (3.07 ± 0.65 mg/g), chlorogenic acid (2.61 ± 0.34 mg/g), caffeic acid (2.57 ± 0.47 mg/g), *p*-coumaric acid (0.52 ± 0.01 mg/g) *p*-hydroxybenzoic acid (0.32 ± 0.02 mg/g). This discrepancy is possibly due to the harsher MAE conditions applied in our study. On the other hand, ASE at higher temperatures and longer exposure times reduced the phenolic diversity in the thyme extract [48]. The highest extraction yields of thyme hydroxycinnamic acids, flavones, and flavonols/flavanones were achieved at 100 °C (5–30 min), while at 150 °C, all yields were negatively affected by the exposure time. Moreover, a significant decrease in extraction yield in the aforementioned study was observed at 200 °C. Therefore, as mentioned above, the cumulative effects of the formation, transformation, and decomposition of phenolic derivatives and non-phenolic compounds at high temperatures should be considered when optimizing extraction conditions.

3.3. Effects of Extraction Parameters on Antioxidant Properties

3.3.1. Antioxidant Properties Evaluated by ABTS Assay

The radical-scavenging activity of wild strawberry leaf extracts against $ABTS^{\bullet+}$ is presented in Table 2 (MAE) and Table 3 (ASE). As can be observed, the reactivity of the extracted compounds toward $ABTS^{\bullet+}$ ranged from 312 to 683 µmol TE/g DW for MAE and from 442 to 627 µmol TE/g DW for ASE. It is worth noting that there are two major differences from the TPC results. First, the range of the results obtained with the two different extraction methods (MAE, ASE) no longer shows such a large deviation, and second, ASE is no longer superior when it comes to the effectiveness of the extracts against $ABTS^{\bullet+}$.

Table 2. Antioxidant properties (using ABTS, DPPH, and FRAP assays) of extracts from wild strawberry leaves obtained by microwave-assisted extraction (MAE) using different combinations of extraction parameters: temperatures of 60, 70, and 80 °C (T60, T70, T80), extraction times of 5 and 10 min (t5, t10), and solvent-to-sample ratios of 20, 30, and 40 mL/g (r20, r30, r40). Data are expressed as mean ± standard deviation. Different letters indicate statistically significant differences according to Kruskal–Wallis test ($p < 0.05$).

MAE	T (°C)	t (min)	R (mL/g)	ABTS (µmol TE/g)	DPPH (µmol TE/g)	FRAP (µmol TE/g)
T60-t5-r20	60	5	20	350 ± 12^{ab}	444 ± 5^{ab}	458 ± 29^{abcd}
T60-t5-r30	60	5	30	368 ± 51^{abc}	539 ± 33^{abc}	536 ± 63^{abcd}
T60-t5-r40	60	5	40	433 ± 12^{abc}	661 ± 32^{bc}	604 ± 79^{abcd}
T60-t10-r20	60	10	20	312 ± 8^{a}	434 ± 14^{ab}	429 ± 12^{abc}
T60-t10-r30	60	10	30	466 ± 5^{abc}	557 ± 20^{abc}	515 ± 49^{abcd}
T60-t10-r40	60	10	40	466 ± 42^{abc}	669 ± 14^{bc}	541 ± 25^{abcd}
T70-t5-r20	70	5	20	392 ± 77^{abc}	477 ± 28^{abc}	427 ± 31^{ab}
T70-t5-r30	70	5	30	371 ± 24^{abc}	545 ± 1^{abc}	360 ± 20^{a}
T70-t5-r40	70	5	40	442 ± 47^{abc}	711 ± 20^{c}	510 ± 73^{abcd}
T70-t10-r20	70	10	20	347 ± 19^{ab}	470 ± 12^{abc}	402 ± 36^{ab}
T70-t10-r30	70	10	30	444 ± 43^{abc}	617 ± 8^{abc}	515 ± 86^{abcd}
T70-t10-r40	70	10	40	440 ± 12^{abc}	697 ± 3^{c}	442 ± 12^{abcd}
T80-t5-r20	80	5	20	471 ± 31^{abc}	352 ± 2^{a}	1834 ± 76^{abcd}
T80-t5-r30	80	5	30	517 ± 28^{bc}	475 ± 7^{abc}	2118 ± 34^{bcd}
T80-t5-r40	80	5	40	681 ± 11^{c}	583 ± 45^{abc}	2389 ± 175^{cd}
T80-t10-r20	80	10	20	413 ± 29^{abc}	345 ± 23^{a}	1666 ± 55^{abcd}
T80-t10-r30	80	10	30	584 ± 32^{bc}	457 ± 1^{abc}	2082 ± 143^{bcd}
T80-t10-r40	80	10	40	683 ± 73^{c}	580 ± 3^{abc}	2461 ± 89^{d}

When observing the results for MAE, a comparison between ABTS and Folin–Ciocalteu assays reveals a similar order of samples in terms of their reactivity in individual tests (also confirmed by correlation analysis, Table 4): the lowest and the highest values are occupied by the same groups of samples in both methods. Among the tested combinations of conditions for MAE, 80 °C and SSR of 40:1 proved to be the most effective (regardless of whether the extraction lasted 5 or 10 min). On the other hand, the minimum efficiency at 80 °C was obtained after 10 min extraction and an SSR of 20:1. The latter did not differ statistically from the majority of treatments carried out at 60 and 70 °C; only one extract (MAE: 60 °C, 20:1 mL/g, 10 min) was found to be significantly less effective, namely, 24%. In addition, a comparison of the trials at the same extraction time and SSR showed that there were no differences between 60 and 70 °C.

Table 3. Antioxidant properties (using ABTS, DPPH, and FRAP assays) of extracts from wild strawberry leaves obtained by accelerated solvent extraction (ASE) using different combinations of extraction parameters: temperatures of 100, 125, and 150 °C (T100, T125, T150), static extraction times of 5 and 10 min (t5, t10), and solvent-to-sample ratios of 20, 30 and 40 mL/g (r20, r30, r40). Data are expressed as mean ± standard deviation. Different letters indicate statistically significant differences according to Kruskal–Wallis test ($p < 0.05$).

ASE	T (°C)	t (min)	R (mL/g)	ABTS (μmol TE/g)	DPPH (μmol TE/g)	FRAP (μmol TE/g)
T100-t5-r20	100	5	20	503 ± 13^{abcd}	514 ± 3^{ab}	758 ± 26^{a}
T100-t5-r30	100	5	30	483 ± 16^{abc}	688 ± 2^{abcd}	809 ± 5^{abc}
T100-t5-r40	100	5	40	509 ± 35^{abcd}	717 ± 8^{abcd}	742 ± 69^{a}
T100-t10-r20	100	10	20	508 ± 7^{abcd}	515 ± 3^{abc}	757 ± 8^{a}
T100-t10-r30	100	10	30	575 ± 8^{abcd}	734 ± 1^{abcd}	953 ± 21^{abc}
T100-t10-r40	100	10	40	496 ± 10^{abcd}	757 ± 4^{abcd}	1057 ± 37^{c}
T125-t5-r20	125	5	20	581 ± 4^{abcd}	514 ± 4^{ab}	860 ± 14^{abc}
T125-t5-r30	125	5	30	602 ± 23^{bcd}	742 ± 4^{abcd}	1045 ± 10^{bc}
T125-t5-r40	125	5	40	627 ± 14^{cd}	839 ± 5^{abcd}	786 ± 19^{ab}
T125-t10-r20	125	10	20	581 ± 5^{abcd}	514 ± 0^{ab}	966 ± 3^{abc}
T125-t10-r30	125	10	30	595 ± 8^{bcd}	756 ± 4^{abcd}	853 ± 18^{abc}
T125-t10-r40	125	10	40	605 ± 13^{cd}	843 ± 2^{bcd}	896 ± 23^{abc}
T150-t5-r20	150	5	20	540 ± 6^{abcd}	512 ± 2^{a}	865 ± 19^{abc}
T150-t5-r30	150	5	30	595 ± 7^{bcd}	754 ± 3^{abcd}	959 ± 15^{abc}
T150-t5-r40	150	5	40	595 ± 4^{bcd}	903 ± 3^{d}	1086 ± 15^{c}
T150-t10-r20	150	10	20	450 ± 5^{ab}	514 ± 1^{abc}	893 ± 9^{abc}
T150-t10-r30	150	10	30	442 ± 7^{a}	706 ± 3^{abcd}	928 ± 13^{abc}
T150-t10-r40	150	10	40	504 ± 9^{abcd}	889 ± 3^{cd}	911 ± 14^{abc}

Table 4. Correlation analysis between total phenolic content (TPC) and antioxidant properties (using ABTS, DPPH, and FRAP assays) of extract from wild strawberry leaves obtained by microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE).

	TPC	ABTS	DPPH	FRAP
TPC		0.894 **	0.261 *	0.790 **
ABTS	0.627 **		0.21	0.798 **
DPPH	0.311 **	0.340 **		−0.297 *
FRAP	0.524 **	0.21	0.373 **	

** Correlation is significant at the 0.01 level (2-tailed); * correlation is significant at the 0.05 level (2-tailed).

The impact of the SSR on the $ABTS^{\bullet+}$ antiradical activity of MAE extracts was also investigated (Table 2). Generally, the lowest values were determined for samples in which the SSR was the lowest. A comparison of SSRs at a constant temperature and time showed the strongest effect of SSR at 80 °C, where the differences in $ABTS^{\bullet+}$ antiradical activity between the minimum and maximum ratios were 45 and 65% for 5 and 10 min extractions, respectively.

The mutual comparison of the MAE experiments performed at the same extraction temperature and ratio showed an insignificant influence of the extraction time. Contrary results were presented for *Garcinia pendunculata* Robx. fruits [49], where the $ABTS^{\bullet+}$ antiradical activity decreased with an increase in irradiation time (4–10 min), while it was not affected by the SSR (10:1–20:1).

As is shown in Table 3, the most rigorous ASE conditions (150 °C, 10 min) applied had a negative effect on the $ABTS^{\bullet+}$ antiradical activity, which is in accordance with a previous finding [50] that severe heat processing (135–160 °C) considerably decreased the $ABTS^{\bullet+}$ antiradical activity of green microalga *Chlorella vulgaris* extracts. Moreover, our extract with the highest TPC (ASE: 150 °C, 10 min, 40:1) expressed only moderate activity in the

ABTS assay. On the other hand, the thermal sensitivity of antioxidants decreased when a shorter extraction time was used. The efficacy of those extracts was very similar to that of all six extracts prepared at 125 °C. Two of them (ASE: 125 °C, 5 min and 10 min, 40:1) had the strongest inhibitory activity against $ABTS^{\bullet+}$ and were quite effective in reducing the Folin–Ciocalteu reagent as well.

Previous research [51] revealed that with the exception of gallic acid (which possesses more than one hydroxyl group), *p*-hydroxycinnamic acids (ferulic > sinapic > *p*-coumaric) are more effective in scavenging $ABTS^{\bullet+}$ than *p*-hydroxybenzoic acids (*p*-hydroxybenzoic ~ vanillic > syringic). This is consistent with our results, as despite the 2.5-fold lower content of syringic acid, the MAE extracts still showed a better ability to scavenge $ABTS^{\bullet+}$ than ASE extracts, possibly due to the 4-fold higher content of gallic acid in the former. As the authors reported, the results (absolute and relative values) of their analysis carried out in phosphate buffer at pH 7.4 differ from those obtained in ethanol. This is in accordance with findings suggesting that the number of electrons exchanged in the Folin–Ciocalteu and ABTS assays depends on the composition of the solvent, the pH of the reaction medium, the duration of the test, and the chemical structure of the antioxidant [52].

In summary, the highest $ABTS^{\bullet+}$ antiradical activity among all 36 experiments tested was obtained with MAE at 80 °C and an SSR of 40:1 after both 5 and 10 min (681 and 683 µmol TE/g DW, respectively), and 5 min should be preferred due to lower energy consumption.

3.3.2. Antioxidant Properties Evaluated by DPPH Assay

The radical-scavenging activity of wild strawberry leaf extracts against $DPPH^{\bullet}$ is presented in Table 2 (MAE) and Table 3 (ASE). As is shown, the reactivity of the extracted compounds toward $DPPH^{\bullet}$ ranged from 345 to 711 µmol TE/g DW for MAE and from 512 to 903 µmol TE/g DW for ASE. The greatest effect of temperature was observed with an SSR of 20:1, where an increase from 60 to 70 °C resulted in a 10% improvement, while a further increase in temperature to 80 °C resulted in a 20% decrease in the extract's reactivity toward $DPPH^{\bullet}$. Therefore, the results obtained by DPPH analysis indicated the different distributions of samples, as observed in the ABTS assay (which was confirmed by correlation analysis, presented in Table 4). While MAE performed at the highest temperature produced the most effective antioxidants against $ABTS^{\bullet+}$, the opposite trend was observed in the DPPH assay. Although the DPPH and ABTS methods are based on the same principle, the activity of particular phenolic molecules toward $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ may be different, as discussed later. According to previous findings [53], the efficiency of optimal MAE conditions differs for two main groups of phenolics that are expected in wild strawberry leaves, with a lower temperature (60 °C) and shorter time (6–9 min) being more convenient for anthocyanin extraction and a higher temperature (70 °C) and longer time (10 min) being preferable for phenolic acids. Last but not least, increasing the extraction temperature leads to rapid cell disruption, which may lead to an increase in impurities in the extracts, thus affecting the antioxidant activity.

In addition, all extracts prepared with the highest SSR (20:1) showed lower $DPPH^{\bullet}$ antiradical activity (from 48 to 68%) than the corresponding extracts prepared with the lowest SSR (40:1). These increased TE values obtained at an increased SSR were similarly demonstrated for extracts from strawberry (*Fragaria* × *ananassa* D.) leaves (MAE: 400 W, 40 s, 20:1–70:1), where the maximum was determined at 60 mL/g [25]. Contrary results (17:1, 30:1, 120:1) were reported for almond skin [23], while the SSR (10:1–20:1) did not have any significant effect on the $DPPH^{\bullet}$ antiradical activity of *Garcinia pendunculata* Robx. extracts [49].

Moreover, in the present study, the irradiation time had no significant impact on $DPPH^{\bullet}$ antiradical activity of wild strawberry leaves (5 and 10 min). In agreement with the results obtained for almond skin (20, 40, 60 s) [23], our observations are not in line with the results reported for *G. pendunculata* Robx. fruits [49], where the $DPPH^{\bullet}$ antiradical activity decreased with increasing irradiation time (4–10 min). A shorter extraction time (10–60 s)

was tested on strawberry (*Fragaria* × *ananassa* D.) leaves (MAE: 300 W, 50:1), for which the highest DPPH$^{\bullet}$ antiradical activity was determined after 40 s [25].

In ASE (Table 3), only a minor effect of temperatures ranging from 100 to 150 °C can be observed in treatments with a constant extraction time and SSR. Meanwhile, extracts produced with an SSR of 20:1 exhibited almost 40% lower antiradical activity (by inhibiting DPPH$^{\bullet}$) than those with an SSR of 40:1. In addition, the impact of the extraction time for ASE extracts was also investigated, and changes amounted to max 10%. Contrary results were reported by others [48], where powdered thyme leaves were extracted with hot water (20:1) at four temperatures, 50, 100, 150, and 200 °C, and at three extraction times, 5, 15, and 30 min. An increase in the extraction temperature from 50 to 200 °C increased the DPPH$^{\bullet}$ antiradical activity by half, whereas different static times with the same temperature regime had no impact on it.

It is worth mentioning that different distributions of ASE extract efficiency were observed in the DPPH method and ABTS method (which is confirmed by the correlation analysis presented in Table 4). The DPPH and ABTS methods are both based on hydrogen-atom or/and single-electron transfer mechanisms. Both reaction pathways can be used simultaneously for deactivation, depending on the properties of the antioxidant and the reaction environment [54,55]. Antioxidants with a simple structure and a reactive group reach equilibrium very fast, while other compounds with more complex structures and possibly multistep action require longer reaction times [56]. However, compounds with similar basic skeletons but different natures of substituents in the ring structures (structural derivatives) can differ in the reaction mechanism that prevails in a particular assay [57]. On the other hand, research performed on caffeic acid and three isomers of its esterified form with quinic acid (caffeoylquinic acid) revealed that all four compounds possess very similar values for bond dissociation enthalpies, proton affinities, electron transfer enthalpies, ionization potentials, and proton dissociation enthalpies, whatever mechanism they follow [54,55]. Those findings are in good agreement with another study, where quite similar antioxidant activities of three caffeoylquinic acid isomers were determined [58]. According to our results, the MAE extract contained higher amounts of caffeic acid and three caffeoylquinic acid isomers (50 mg/100 g) than the ASE extract (47 mg/100 g) and expressed better ABTS$^{\bullet+}$, but not DPPH$^{\bullet}$, antiradical activity. The same research [58] showed that dicaffeoylquinic acids possessed significantly better antioxidative activities against DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ than caffeoylquinic acids. In our study, we found comparable amounts of dicaffeoylquinic acids in both extracts, but due to their low amounts (<2 mg/100 g), they probably did not have a major influence on the overall antioxidant activity of the MAE and ASE extracts. However, it is worth considering that unfavorable extraction conditions could lead to the formation of derivatives and isomers of the original compounds. In our case, the reactivity of the extracts against DPPH$^{\bullet}$ was determined to be higher than that against ABTS$^{\bullet+}$. Different antiradical activity of the same compounds against DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ was confirmed before [54,56]. As documented earlier [57], features such as electron donation and hydrogen supply due to substitutions in the structure of the compounds were found to play an important role in their DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ antiradical activity. According to the outcomes of a previous study [59], the highest DPPH$^{\bullet}$ antiradical activity in ethanol was determined for phenolics containing more hydroxyl groups (dihydrocaffeic acid, rosmarinic acid, caffeic acid), followed by monophenolics with methoxy substituents (sinapic acid, ferulic acid). Simple phenolics without an aromatic ring substituent (coumaric acids, cinnamic acid) were almost inactive toward DPPH$^{\bullet}$, which is in contrast to ABTS$^{\bullet+}$, as mentioned earlier. Another comparative study [56] indicated that in the DPPH assay (performed in methanol), a molecule of gallic acid and quercetin showed similar reactivity to Trolox, while ferulic acid, catechin, and ascorbic acid were not half as effective as the Trolox molecule. In the ABTS assay (applied in methanol, radical solution diluted with ethanol), on the other hand, these standards showed reactivity about 2- to 3-fold higher than Trolox molecules, with the exception of ascorbic acid (which achieved similar reactivity to Trolox).

In summary, the highest DPPH• antiradical activity among all 36 experiments tested was obtained after 5 min ASE at 150 °C and an SSR of 40:1 (903 µmol TE/g DW).

3.3.3. Antioxidant Properties Evaluated by FRAP Assay

The ferric reducing antioxidant power of wild strawberry leaf extracts is presented in Table 2 (MAE) and Table 3 (ASE). The reactivity of the extracted compounds in the FRAP assay ranged from 360 to 2461 µmol TE/g DW for MAE and from 742 to 1086 µmol TE/g DW for ASE. It is worth noting that the interval of results for MAE extracts is considerably wider (6.8-fold range) compared to the results obtained for ASE extracts within the same method as well as compared to MAE in all other methods.

With respect to MAE, the data show that there were no major differences in the reducing power of the extracts when prepared at 60 or 70 °C. At 80 °C, on the other hand, the potency was enormously increased. In addition, at 80 °C, a higher SSR resulted in more effective extracts, namely, 15% (SSR 30:1) and 30% (SSR 40:1) after 5 min of extraction, while an even higher increase was observed after 10 min, namely, 25% (SSR 30:1) and 48% (SSR 30:1). On the other hand, the impact of extraction time at a constant temperature and SSR was irrelevant. For comparison, 60 mL/g (MAE: 400 W, 40 s, 20:1–70:1) was the optimal SSR for the FRAP of strawberry (*Fragaria* × *ananassa* D.) leaves, while the highest reducing power was determined after 30 s (MAE: 300 W, 50:1, 10–60 s) [25]. A similar finding was reported [60] for banana peel (MAE: 720 W, 6 min), where the FRAP decreased by a quarter as the SSR decreased from 50 to 25 mL/g. On the other hand, the same study (MAE: 720 W, 50:1) indicated that the FRAP increased with the extraction time up to 4 min, after which it decreased.

Back to our observations, the trend was less pronounced in the case of ASE. The least effective samples included those extracted at 100 °C for 5 min or with a 20:1 ratio. In contrast, the sample extracted at 100 °C for 10 min with an SSR of 40:1 was among the most effective. Compounds extracted for 10 min at 125 or 150 °C displayed a moderate effect. A higher SSR resulted in the higher reducing power of the compounds obtained after 5 min extraction at 150 °C, namely, for 11 and 25%. The latter is not in line with results derived from the extraction of phenolics from thyme leaves [48], where a simpler trend was observed. In the cited work, at an SSR of 20:1, very high extraction temperatures, namely, 150 and 200 °C, resulted in a significantly improved FRAP in comparison with 50 and 100 °C, with no effect of the static time (5–30 min) within the same temperature regime.

In summary, the best reducing properties among all 36 samples were determined for extracts prepared with MAE at 80 °C and an SSR of 40:1 (2389 and 2461 µmol TE/g DW after 5 and 10 min, respectively), and 5 min should be preferred due to lower energy consumption.

The antioxidant properties are expressed as Trolox equivalent in the DPPH, ABTS, and FRAP methods to enable the direct comparison of the results. FRAP is based on an electron transfer mechanism; since the ferric 2,4,6-tripyridyl-s-triazine complex reacts with an antioxidant compound, a single electron is transferred to the ferric ion, converting it into ferrous tripyridyltriazine [61]. According to our findings, some similarity exists in the reactivity of extracts between the FRAP and ABTS methods. Further, the results revealed that the reducing power of the prepared extracts strongly differs from their ability to scavenge DPPH•. Based on the literature [62], the reducing power of phenolics is greatly pronounced in the presence of an additional hydroxyl group (hypogallic acid, gentistic acid) located either in vicinal positions or on opposite sides of the ring (in *ortho* or *para* position to each other). Phenolics with more than two hydroxyl groups (gallic acid) expressed slightly lower efficiency, while the presence of two methoxy groups (syringic acid, sinapic acid) demonstrated only moderate activity. Methylated phenolic acid (ferulic acid, vanillic acid) derivatives were less efficient compared to their nonmethylated counterparts (caffeic acid, protocatechuic acid) due to the decreasing number of active electron- and hydrogen-donating groups. Researchers found that benzoic acid (protocatechuic acid, vanillic acid) demonstrated mostly lower efficiency than their counterparts derived from cinnamic

acid derivatives (caffeic acid, ferulic acid) due to an increase in the carboxylic group's electron-withdrawing effect on radical delocalization. Monohydroxylated hydroxybenzoic (*p*-hydroxybenzoic acid) and hydroxycinnamic (*p*-, *o*-, *m*-coumaric acids) acids expressed the lowest activities in the cited study.

3.4. Correlation between the Contents of Phenolics and Their Different Antioxidant Properties

The correlation coefficients between the results obtained by different assays are shown in Table 4. A strong significant correlation was found between the TPC, ABTS, and FRAP values for MAE extracts. Thus, the amount of extracted phenolics may have a role in the observed antioxidant properties of wild strawberry leaf samples.

In addition, compounds that were able to scavenge $ABTS^{\bullet+}$ were capable of reducing the yellow ferric complex to a blue ferrous complex as well. In contrast, a poor negative correlation between the DPPH and FRAP values suggests that compounds with greater $DPPH^{\bullet}$ antiradical activity were less efficient in reducing ferric ions. On the other hand, only a poor to moderate positive interrelation among studied responses was observed for ASE extracts, probably due to the greater diversity of the extract composition and the presence of non-phenolic compounds. Despite these assays being based on similar chemical mechanisms, the relatively poor correlation observed between most of them indicates that the same ASE parameter has a different effect on the phenolic antioxidants in wild strawberry leaves. Interestingly, a recent study on *M. oleifera* leaves [41] has shown that TPC and antioxidant activity measured by ABTS (the ABTS test allows the measurement of both hydrophilic and lipophilic compounds) was higher for extracts obtained under MAE conditions than under ASE ones, while no significant differences between the DPPH results (the DPPH test is limited to hydrophilic antioxidants) by both extraction methods were observed. A similar trend was observed in this study. However, the results suggest that this type of comparison between the two techniques is highly dependent on the extraction conditions chosen. Even a slight change in one parameter can have a significant impact on the extraction performance.

4. Conclusions

Wild strawberry leaves, which, after the collection of the fruits, are generally thrown away, could be an interesting byproduct rich in diverse classes of phenolics. In an attempt to utilize their antioxidant properties, this study evaluated the influence of extraction conditions using two green extraction techniques. The optimal MAE procedure (80 °C, 5 min, SSR 40:1) was found to be more specific for the isolation of phenolics that are able to scavenge $ABTS^{\bullet+}$ radicals and reduce ferric ions to ferrous ions, while the optimal ASE conditions (150 °C, 5 min, SSR 40:1) enabled the highest TPC and produced extracts with the highest $DPPH^{\bullet}$ antiradical activity. To summarize, a change in temperature or/and in the SSR had a significant impact on the extraction performance of MAE and ASE, while the extraction time was less relevant. In order to explore the application potential, the yields of different phenolics under optimal conditions were compared. UPLC/MS-MS analysis showed a much higher (43%) content of phenolics (the summation of 54 individual phenolics) in the ASE extract than in MAE, both obtained under optimal conditions. ASE resulted in higher yields of proanthocyanidins (397.81 ± 11.25 mg/100 g), flavonols (1446.94 ± 40.93 mg/100 g), flavan-3-ols (108.03 ± 3.06 mg/100 g), and flavones (27.73 ± 0.78 mg/100 g), while MAE was more efficient for the extraction of phenolic acids (484.71 mg/100 g). Our study is the first comprehensive examination of wild strawberry leaves' phenolic profile. In general, procyanidin B1, myricetin, quercetin, kaempferol, rutin, quercetin-3-glucoronide, quercetin-3-glucoside, myricetin-3-*O*-rhamnoside, kaempferol-3-glucoronide, epicatechin, luteolin, gallic acid, *p*-hydroxybenzoic acid, chlorogenic acid, and 5-*O*-galloylquinic acid were the major phenolics, regardless of the extraction technique. Many of the compounds detected in the leaves have previously been related to various biological effects, and further knowledge of the precise phenolic profile could expand their use in the cosmetic, nutraceutical, and pharmaceutical industries. Although each extraction technique has its own advantages and

disadvantages, MAE and ASE can also be combined to achieve the lower degradation of metabolites with improved antioxidant properties, which is associated with sustainability and environmental benefits.

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