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Microgreen-Based Juices: UHPLC Q-ToF MS Metabolomic Analysis, Antioxidant Properties, and Sensory Acceptability

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Abstract

The aim of this study was to develop novel and sensory-acceptable microgreens–apple juices and characterize them using UHPLC Q-ToF MS-based metabolomic analysis. The obtained juices showed reduced sugar content and mild acidity compared with the control apple juice. Untargeted analysis revealed 71 compounds, including phenolics, betalains, and one glucosinolate. Semi-quantification confirmed a high content of sinapic acid and its derivatives and hydroxybenzoic acid in broccoli–apple juice, as well as a predominant amount of phenolic acid diglycosides and hydroxycinnamoyl–isocitric acid derivatives in amaranth–apple juice. Apigenin C-glycosides were the main phenolics in red beet–apple juice, with the highest content of cytisoside derivatives. All microgreen-based juices contained apple-derived flavan-3-ols, procyanidins, dihydrochalcones, and certain flavonols. The formulated juices exhibited promising antioxidant potential evaluated by several screening assays (TPC, DPPH, ABTS, and FRAP), consistent with their phytochemical profiles, while differences among them may be linked to the amounts of certain bioactive molecules, mainly derived from microgreens. Moreover, these juices showed high overall quality and consumer acceptability, successfully masking typical microgreens flavors. Overall, these cold-pressed microgreen–apple juices can be classified as novel, low-calorie, and highly sensory-acceptable beverages, containing diverse bioactive compounds from both apple and microgreens; however, further in vitro and in vivo evidence is needed to support claims regarding their functionalities.

Keywords: microgreens–apple juices; metabolomic analysis; UHPLC Q-ToF MS; broccoli microgreens; amaranth microgreens; red beet microgreens; antioxidant properties; sensory properties

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1. Introduction

Microgreens are considered among the "fastest-growing" functional products on the global market due to their abundance of bioactive constituents and their nutritional value, the growing trend of indoor urban farming, and their efficient, low-cost production with

minimal environmental impact [1,2]. Microgreens, particularly those from the Brassicaceae and Amaranthaceae families, are recognized as a promising food for the future due to their rich composition of biologically active constituents, including sulfur-containing phytochemicals (glucosinolates and isothiocyanates), betalains, and various phenolic compounds [3–8], which exert beneficial effects on human health. For example, microgreens from the Brassicaceae family exhibit high bioavailability of bioactive compounds [9,10] and may serve as a preventive agent against colon cancer [11]. Microgreens or sprouts are most often consumed raw in salads; however, recent studies have also explored their use in the production of healthy juices and beverages [3,12–15] and their incorporation into bakery, confectionery, and other food products [16]. Nevertheless, microgreen and sprout juices have only recently gained attention and remain insufficiently studied. Preliminary studies have investigated juices derived from broccoli, red beet, amaranth [3,12], *Alternanthera sessilis* [17], and wheatgrass [18]. In addition, cold-pressed microgreen and sprout juices have demonstrated strong antioxidant potential [3,18,19], as well as potential anticancer [13] and anti-obesity effects [12], and protective properties against oxidative stress-related diseases [14]. Additional studies are needed to gain a deeper understanding of their bioactive compound profiles and to confirm their functional properties. Moreover, beverages derived from microgreens often exhibit reduced consumer acceptability due to distinctive herbal, grassy, astringent, and sulfur-like sensory characteristics [3,20,21]. To address these limitations, encapsulation techniques can be applied to mask undesirable flavors [22], or microgreen juices can be blended with fruit juices to improve sensory acceptability [23–25]. Although recent studies have explored the development of ready-to-serve microgreen-fruit beverages [23,25], this area remains insufficiently investigated and requires novel formulations, advanced characterization, and further preclinical studies (in vitro and in vivo experiments).

On the other hand, cold-pressed apple juice is a widely consumed and globally produced beverage [26], appreciated for its balanced sweetness, mild acidity, and fresh aroma. In addition, apples are characterized by a high phenolic content [27,28], which can selectively migrate into the juice during maceration and pressing [29–31]. The consumption of cloudy apple juice has been linked to improved cardiovascular health, as well as a reduction in cancer risk, neurodegenerative diseases, and other chronic diseases [32]. For the mentioned reasons, apple juice is commonly used as a base in the formulation of various food products, mixed fruit juices, and juice blends, contributing to improved functional properties, nutritional value, and sensory acceptability of these products [33,34]. To the authors' knowledge, blends of apple and microgreen juices have not yet been formulated and systematically analyzed. Considering that apple is a rich source of flavonoids not typically present in broccoli, red beet, or amaranth microgreens [3,6], their juice blends may provide a more diverse profile of bioactive compounds and improved sensory acceptability. Accordingly, this study aimed to develop microgreen–apple juice blends by combining cold-pressed broccoli, amaranth, and red beet microgreen juices with cold-pressed apple juice, in order to obtain novel beverages with enhanced antioxidant properties and sensory characteristics. To characterize the obtained microgreen–apple juice blends, untargeted UHPLC Q-ToF MS analysis combined with multivariate (PCA and HCA) statistical analysis was performed, while the distribution of phenolic compounds was evaluated through quantification and semi-quantification.

2. Materials and Methods

2.1. Production of Cold-Pressed Microgreen–Apple Juices

Cold-pressed microgreen juices were prepared as described in our previous study by Belošević, Milinčić, Gašić, Kostić, Salević-Jelić, Marković, Đorđević, Lević, Pešić and

Nedović [3]. Fresh microgreens were sourced from a local microgreens producer (Plantica company, Belgrade, Serbia) in plastic containers with a commercial peat-based substrate. The microgreens were harvested by cutting above ground with sterilized scissors, and subsequently washed with tap water to remove residual impurities, such as soil, seeds, and dust. Apples (Golden Delicious) were purchased from a local market and washed and chopped using a sterilized knife prior to juice preparation. The prepared microgreens and apples were processed using a super-slow juicer (Angel Juicer 8500, Angel Co., Ltd., Busan, Republic of Korea). In order to reduce specific sensory attributes of microgreens and maintain a high percentage of microgreen juice in the blends, this experimental study mixed microgreen–apple juices in a specific ratio of 51:49 (v/v). It was also taken into account that apple juice is commonly used as a base in commercial juice blends, and a proportion of around 50% is often considered appropriate for balancing sensory characteristics in such juice blends. However, it should be noted that the prepared microgreen–apple blends (51:49 v/v) represent a single point in a broader optimization space for each of the prepared juices: red beet microgreens–apple juice (RB-AJ); broccoli–apple juice (BC-AJ); and amaranth–apple juice (AM-AJ). The prepared juices were packaged in plastic bottles and stored at −20 °C until further experimentation.

2.2. Proximate Composition of Cold-Pressed Microgreen–Apple Juices

The total soluble solids (°Bx) were determined using a digital refractometer (ATC 0–32 Brix, Huixia Supply Co., Ltd., Fuzhou, China), while pH values were determined using a digital pH meter (WTW inoLab, Weilheim, Germany).

2.3. UHPLC Q-ToF MS Analysis of Cold-Pressed Microgreen–Apple Juices

Prior to chromatographic analysis, both the apple (control) juice and microgreen–apple juice blends (RB-AJ, BC-AJ, and AM-AJ) were subjected to solid-phase extraction using SPE cartridges (CLEAN-UP^{R}, C_{18} extraction columns, unendcapped-PKG50, UCT, Bristol, UK). This step was performed to eliminate sugars and impurities and to concentrate the bioactive compounds present in the juice samples. The SPE cartridges were first conditioned by sequential rinsing with 5 mL of methanol (CARLO ERBA Reagents GmbH, Cornaredo, Italy), then by 5 mL of Milli-Q water obtained using purification system BBPS-508 (Biolab Scientific, Markham, ON, Canada). Subsequently, 2.5 mL of the juice sample was loaded onto the conditioned cartridge and allowed to pass through. After sample percolation, the cartridge was further washed with Milli-Q water. The retained bioactive compounds were subsequently eluted with 1 mL of acidified methanol (methanol containing 0.1% HCl). The eluates were filtered through 0.45 µm syringe filters and used for subsequent analysis.

The identification and characterization (untargeted analysis) of the bioactive compounds present in the apple juice and microgreen–apple juice blends were conducted using high-performance liquid chromatography (Agilent 1290 Infinity system), coupled with a quadrupole time-of-flight mass spectrometer (6530C Q-ToF-MS, Agilent Technologies, Santa Clara, CA, USA), using the same Zorbax RRHD Eclipse Plus C18 column (2.1 × 50 mm, 1.8 µm) (Agilent Technologies, Santa Clara, CA, USA), UHPLC method, ESI operating parameters, and Auto MS/MS settings as previously reported and described by Milinčić et al. [35].

Agilent MassHunter software (Qualitative Analysis, version 10.0, Agilent Technologies, Santa Clara, CA, USA) and MS-DIAL (version 4.60) (http://prime.psc.riken.jp/, (accessed on 17 May 2026)) were employed for spectral screening and extraction of relevant data (including retention time, exact monoisotopic mass, and peak area), as well as for data processing, evaluation, and visualization. Compound identification was performed tentatively based on accurate monoisotopic masses and characteristic fragmentation patterns, and validated against reference standards (labeled compounds) and the literature

data, as reported by Belošević, Milinčić, Gašić, Kostić, Salević-Jelić, Marković, Đorđević, Lević, Pešić, and Nedović [3]. The CAS SciFinder^n (https://scifindern.cas.org/) and PubChem (https://pubchem.ncbi.nlm.nih.gov/) databases were utilized to support the identification process by providing information on molecular formulas and chemical structures. To visualize relationships within the metabolomic datasets, identify discriminant metabolites (compounds), and provide a more rigorous comparison among juices, the metabolomic data (peak areas) were analyzed by multivariate unsupervised statistical methods: principal component analysis (PCA) and hierarchical cluster analysis (HCA). The PCA biplot and heatmap were generated using Q-ToF MS peak area data (exported from MS DIAL (ver. 4.60) software (http://prime.psc.riken.jp/, accessed on 17 May 2026)) for all identified compounds. A PCA biplot simultaneously displays both observations and features from a PCA, illustrating how individual samples (juices) relate to or differ from one another and which original variables (bioactive compounds) contribute to those differences or similarities. PCA was performed using Unscrambler X software (version 10.4; CAMO AS, Trondheim, Norway). Hierarchical Cluster Analysis was performed using Spearman's correlation-based agglomeration method in Morpheus software version 5.5.1 (https://software.broadinstitute.org/morpheus (accessed on 17 May 2026)). The input variables were independently normalized using min–max scaling for each compound.

Tentatively identified phenolic compounds through untargeted analysis were also quantified or semi-quantified using structurally similar and available standards. Taking this into account, the concentrations of individual phenolic compounds within each class were expressed in equivalents of selected standards per 100 mL of juice, using protocatechuic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, quercetin, procyanidin B2, phloretin, and isovitexin as reference standards. Standard solutions of phenolic compounds, prepared at various concentrations, were analyzed under identical conditions to those applied for the samples. The validation parameters for all standards used in the quantification and semi-quantification of phenolic compounds are provided in Table S1. This table includes regression equations ($Y = aX \pm b$) along with corresponding parameters, such as the coefficient of determination (R^2), correlation coefficient (r), linearity range, limit of detection (LOD, defined at a signal-to-noise ratio of 3:1), and limit of quantification (LOQ, defined at a signal-to-noise ratio of 10:1). Peak areas for each compound were compared across samples after normalization and consistent processing conditions, and subsequently exported from MS-DIAL software.

2.4. Total Phenolic Content (TPC) and Antioxidant Properties of Cold-Pressed Microgreen–Apple Juices

All microgreen–apple juices were prepared for spectrophotometric assays by centrifugation at 9000× *g* for 12 min to remove the solid fraction, and the collected supernatant was used for further analysis. The total phenolic content (TPC) of the microgreen– apple juices was evaluated using the Folin–Ciocalteu spectrophotometric assay, according to a previously described assay [3], and expressed as mg equivalents of gallic acid per 100 mL of juice (mg GAE/100 mL).

The antioxidant capacity of microgreen-based juices was evaluated using previously described screening methods [3], known as the Ferric Reducing Antioxidant Power (FRAP) assay, DPPH$^\bullet$ free radical scavenging assay, and ABTS$^{\bullet+}$ radical cation scavenging assay. Trolox was used as the reference standard for all applied antioxidant assays, and the results were presented in mg of Trolox equivalents per 100 mL of juice (mg TE/100 mL).

2.5. Sensory Analyses of Cold-Pressed Microgreen–Apple Juices

Sensory evaluation of microgreen-based juices in terms of overall quality and consumer acceptance was conducted by trained evaluators employed at the Faculty of Agriculture [3],

University of Belgrade, who were also regular consumers of cold-pressed fruit and vegetable juices. The sensory evaluation was performed in accordance with the Code of Professional Ethics of the University of Belgrade (Senate of the University of Belgrade, 2016) and the Serbian Law on Personal Data Protection (Official Gazette of the Republic of Serbia, 87/2018). Based on the exemption statement issued by the Ethics Committee of the Faculty of Agriculture, University of Belgrade, Serbia (No. 22/268, issued on 26 May 2026), this study did not require formal ethical approval, since no ethically sensitive procedures were involved. In addition, sensory testing was carried out in a controlled environment following ISO standards for sensory analysis, including ISO 8589:2007 [36] and ISO 11136:2014 [37]. All evaluators were informed that their responses would remain confidential and would be used solely for research purposes, and written informed consent was obtained from all participants. They were also informed of their right to withdraw from the study at any time. The microgreen–apple juices used in the study were safe for sensory evaluation. Samples were served in transparent plastic cuvettes coded with random three-digit numbers. Between samples, evaluators rinsed their palate with water and consumed unsalted crackers to neutralize taste perception.

The quality rating method was used to assess the overall quality of microgreen–apple juices. A categorical scale ranging from 0 (unsatisfactory quality) to 5 (excellent quality) was used. Since not all sensory attributes contribute equally to overall quality, weighting coefficients were applied to the appearance (1), odor (6), texture (5), and taste (8), and used to calculate the overall quality score for each evaluator. Sensory evaluation was carried out by 6 trained evaluators, trained in accordance with ISO 8586:2023 [38], employed at the Faculty of Agriculture. Prior to evaluation, assessors were trained in sensory descriptors and terminology.

The hedonic test was used to evaluate each sample individually based on sensory attributes and overall acceptability, rather than comparing or ranking products against one another. To ensure an independent evaluation, consumers assessed each sensory attribute individually (appearance, texture, taste, odor, and overall acceptability), without comparing samples, while additional comments about sensations or product attributes were recorded in the comment section. Consumer acceptance was evaluated by 55 consumers using a 9-point hedonic scale, where 1–4 indicated dislike, 5 indicated neither like nor dislike, and 6–9 indicated liking. Participants were selected based on regular consumption of fruit and vegetable juices and screened for basic organoleptic sensitivity. The numerical data obtained from the hedonic test were expressed as mean values in a radar diagram. Since the assumptions of normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) were not met for these datasets, the non-parametric Kruskal–Wallis test was used to evaluate differences among the juices for each sensory attribute.

2.6. Statistical Analyses

Results for proximate composition (n = 3), spectrophotometric assays (n = 3), and sensory evaluation were expressed as the mean values ± standard deviation. The statistical test used to evaluate differences among juices was selected based on the specific characteristics of the datasets, including sample size, normality, and homogeneity of variance. However, hypothesis tests such as the Shapiro–Wilk test (normality) and Levene's test (homogeneity of variance) may have limited statistical power at small sample sizes (three juice samples (n = 3) for spectrophotometric assays and six juice samples (n = 6) for sensory quality scores). Consequently, these tests may fail to reliably detect deviations from the underlying assumptions. For the mentioned reasons, significant differences among the juices were determined using the Kruskal–Wallis test ($p < 0.05$), followed by Dunn's post hoc test (GraphPad Prism 6, San Diego, CA, USA). The Kruskal–Wallis test, a non-parametric alternative to one-way ANOVA, does not assume normality or homogeneity of variance, making it a more appropriate and reliable choice for these datasets.

3. Results and Discussion

3.1. Proximate Composition of Cold-Pressed Microgreen–Apple Juices

The results for the proximate compositions of the control apple juice and microgreen–apple juices are presented in Table 1.

Table 1. Proximate compositions of cold-pressed microgreen–apple juices.

Juices	Ratio %	pH	TSS (°Bx)
AM-AJ	51:49	3.82 ± 0.02 [a,b]	7.0 ± 0.06 [a]
BC-AJ	51:49	3.97 ± 0.01 [a]	7.2 ± 0.06 [a]
RB-AJ	51:49	3.91 ± 0.03 [a,b]	7.0 ± 0.06 [a]
AJ	100	3.53 ± 0.02 [b]	13.0 ± 0.10 [a]

Abbreviations: "AJ", apple juice (control juice); "RB-AJ", red beet microgreens–apple juice; "BC-AJ", broccoli microgreens–apple juice; "AM-AJ", amaranth microgreens–apple juice. The results were presented as the mean values ± standard deviation. The different small letters in the same column denote a significant difference, according to Dunn's test, $p < 0.05$.

The pH value of the cold-pressed apple juice (AJ) was the lowest (3.53 ± 0.02), likely due to the presence of organic acids, particularly malic acid, which strongly influences acidity [39], compared with the newly developed microgreen–apple juices: AM-AJ (3.82 ± 0.02), RB-AJ (3.91 ± 0.03), and BC-AJ (3.97 ± 0.01). In our previous study [3], cold-pressed microgreen juices from broccoli, amaranth, and red beet exhibited higher pH values (pH ≈ 5.96, 6.52, and 6.44, respectively) than those observed in the present study. Therefore, it can be concluded that the addition of apple juice decreased pH values and contributed to a slightly more acidic taste. On the other hand, a lower pH may be advantageous in terms of shelf life, as it can contribute to extended microbial stability [40]. The results obtained in this study are consistent with those reported by Senthilnathan and Muthusamy [17], who analyzed untreated *Brassica juncea* L., microgreen juice mixed with ginger, cinnamon, lemon, pineapple, honey, lemongrass, and mint (4.02). In contrast, the addition of apple juice increased total soluble solids (TSS), giving higher values then these reported in cold-pressed microgreen juices (TSS ≈ 2) [3]. The microgreen-based juices showed similar TSS values, ranging from 7.0 °Bx (AM-AJ and RB-AJ) to 7.2 °Bx (BC-AJ), while the AJ exhibited significantly higher values (13 °Bx). These values were lower than those reported for *Alternanthera sessilis* (37.76 °Bx) and *Brassica juncea* L. microgreen juices (11.3 °Bx) [17,41]. These differences in TSS may be explained by variations in formulation, particularly the use of sweeteners, such as honey, and additional ingredients like lemon, pineapple, and ginger. Overall, the addition of apple juice influenced the physicochemical properties by decreasing pH and increasing TSS, and these microgreen–apple juices can be considered decreased-calorie beverages.

3.2. Untargeted Metabolomic Profiling of Cold-Pressed Microgreen–Apple Juices

Untargeted UHPLC–Q-ToF MS analysis was performed to identify all bioactive compounds in the microgreen–apple juice blends, contributing to the promotion of these beverages, the explanation of some processing-related transformations, and the understanding of differences in their antioxidant properties, as well as functional properties in future studies. Untargeted analysis revealed a total of 71 compounds in the analyzed apple and microgreen–apple juices, which mainly belong to phenolic compounds (phenolic acids and flavonoids) (61 compounds), followed by betalains (nine compounds) and a single glucosinolate (Table 2). However, the prepared microgreen–apple juices exhibited distinct phytochemical profiles depending on the bioactive compounds derived from the red beet, broccoli, and amaranth microgreens. Apple juice was also analyzed to verify phenolic compounds originating from apple and to track their distribution in microgreen–apple juice blends.

Table 2. UHPLC Q-ToF MS identification and characterization of bioactive compounds in apple (AJ) and various microgreen–apple juices (RB-AJ, BC-AJ, and AM-AJ).

No.	Compound Names	t_R	Formula	Calculated Mass	*m/z* Exact Mass	Δ mDa	MS2 Fragments (%, Main Fragment)	Juice Samples			
								AJ	RB-AJ	BC-AJ	AM-AJ
					Phenolic acids						
					Hydroxybenzoic acids and derivatives						
1	**Hydroxybenzoic acid**	3.94	$C_7H_5O_3^-$	137.0239	137.0252	−1.30	**108.0233(100)**, 119.0149	+	+	+	+
2	**Hydroxybenzoic acid hexoside is. I**	1.68	$C_{13}H_{15}O_8^-$	299.0772	299.0777	−0.46	**137.0248(100)**	+	+	+	+
3	**Hydroxybenzoic acid hexoside is. II**	5.73	$C_{13}H_{15}O_8^-$	299.0772	299.0787	−1.46	**137.0252(100)**	**NF**	+	+	+
4	**Hydroxybenzoic acid pentosyl hexoside**	3.49	$C_{18}H_{23}O_{12}^-$	431.1195	431.1215	−2.00	**137.0251(100)**, 281.0693	**NF**	**NF**	**NF**	+
5	**Protocatehuic acid ***	2.30	$C_7H_5O_4^-$	153.0193	153.0197	−0.37	**108.0229(100)**, 109.0294	+	+	+	+
6	**Dihydroxybenzoic acid pentoside**	4.58	$C_{12}H_{13}O_8^-$	285.0616	285.0643	−2.71	**108.0231(100)**, 109.0291, 152.0117, 153.0164	**NF**	+	**NF**	**NF**
7	**Dihydroxybenzoic acid hexoside**	2.28	$C_{13}H_{15}O_9^-$	315.0722	315.0729	−0.74	**108.0228(100)**, 109.0291, 152.0115, 153.0181	+	+	+	+
8	**Acetyl–vanillic acid**	11.25	$C_{10}H_9O_5^-$	209.0455	209.0479	−2.35	**149.0248(100)**, 193.0136, 167.0379, 123.0460, 105.0353	**NF**	**NF**	+	**NF**
9	**Vanillic acid hexoside**	3.02	$C_{14}H_{17}O_9^-$	329.0878	329.0890	−1.19	**108.0224(100)**, 123.0457, 152.0117, 167.0339	+	+	+	+
10	**Vanillic acid pentosyl hexoside**	4.43	$C_{19}H_{25}O_{13}^-$	461.1301	461.1334	−3.33	**167.0374(100)**, 123.0461, 152.0123, 329.0901	**NF**	**NF**	**NF**	+

Table 2. *Cont.*

No.	Compound Names	t_R	Formula	Calculated Mass	*m/z* Exact Mass	Δ mDa	MS2 Fragments (%, Main Fragment)	Juice Samples			
								AJ	RB-AJ	BC-AJ	AM-AJ
11	**Vanilloside**	4.09	$C_{14}H_{17}O_8^-$	313.0929	313.0938	−0.91	**107.0511(100)**, 123.0459, 135.0489, 151.0390, 153.0467	+	NF	NF	NF
12	**Gallic acid hexoside**	1.68	$C_{13}H_{15}O_{10}^-$	331.0665	331.0692	−2.70	**168.0061(100)**, 169.0120, 125.0249, 149.9961, 313.0559	+	+	+	+
13	**Syringic acid hexoside**	4.98	$C_{15}H_{19}O_{10}^-$	359.0984	359.1019	−3.56	**138.0328(100)**, 123.0100, 153.0566, 166.9975, 182.0212, 197.0451	+	NF	NF	+
14	**Shikimoylquinic acid hexoside**	2.25	$C_{22}H_{23}O_{15}^-$	527.1037	527.1061	−2.40	**191.0555(100)**, 353.0837, 173.0272	+	+	NF	+
Hydroxycinnamic acids and derivatives											
Coumaric acid derivatives											
15	**Coumaric acid hexoside**	6.27	$C_{15}H_{17}O_8^-$	325.0923	325.0945	−2.20	**119.0504(100)**, 145.0286, 163.0388	+	+	+	+
16	**Coumaroylquinic acid is. I**	5.74	$C_{16}H_{17}O_8^-$	337.0929	337.0937	−0.81	**119.0511(100)**, 127.0428, 155.0351, 163.0398, 173.0450, 191.0552, 111.0460	+	+	+	+
17	**Coumaroylquinic acid is. II**	6.88	$C_{16}H_{17}O_8^-$	337.0929	337.0944	−1.52	**173.0453(100)**, 119.0510, 137.0239, 155.0348, 163.0398, 191.0557	+	+	+	+
18	**Coumaroyl–(*iso*)citric acid**	7.48	$C_{15}H_{13}O_9^-$	337.0560	337.0591	−3.10	**111.0101(100)**, 119.0510, 129.0207, 154.9999, 173.0100, 191.0197	NF	NF	NF	+
Caffeic acid derivatives											

Table 2. *Cont.*

No.	Compound Names	t_R	Formula	Calculated Mass	*m/z* Exact Mass	Δ mDa	MS2 Fragments (%, Main Fragment)	Juice Samples			
								AJ	RB-AJ	BC-AJ	AM-AJ
19	**Caffeic acid hexoside**	5.39	$C_{15}H_{17}O_9^-$	341.0878	341.0896	−1.82	**161.0245(100)**, 133.0313, 135.045, 179.0354	+	+	+	+
20	**Caffeoylquinic acid is. I**	4.37	$C_{16}H_{17}O_9^-$	353.0878	353.0903	−2.49	**191.0558(100)**, 135.0453, 155.0337, 161.0245, 173.0456, 179.0345	+	+	+	+
21	**Chlorogenic acid *** (3-Caffeoylquinic acid)	6.19	$C_{16}H_{17}O_9^-$	353.0878	353.0893	−1.49	**191.05535(100)**, 127.0407, 135.0452, 161.0242, 173.0454, 179.0341	+	+	+	+
22	**Caffeoylquinic acid is. III**	6.80	$C_{16}H_{17}O_9^-$	353.0878	353.0892	−1.39	**191.0551(100)**, 127.0408, 161.0244, 173.0452	+	+	+	**NF**
23	**Methyl–caffeoylquinate**	7.50	$C_{17}H_{19}O_9^-$	367.1029	367.1040	−1.10	**135.0474(100)**, 179.0361, 161.0247, 191.0554	+	+	+	**NF**
24	**Caffeoyl–(*iso*)citric acid**	6.81	$C_{15}H_{13}O_{10}^-$	353.0509	353.0548	−3.90	**111.0100(100)**, 129.0204, 135.0458, 154.9993, 173.0098, 179.0353	**NF**	**NF**	**NF**	+
					Ferulic acid derivatives						
25	**Feruloyl–(*iso*)citric acid**	7.62	$C_{16}H_{15}O_{10}^-$	367.0671	367.0703	−3.23	**111.0102(100)**, 129.0206, 154.9999, 134.0382, 149.0613, 173.0095, 193.0510	**NF**	**NF**	**NF**	+
					Sinapic acid and derivatives						
26	**Sinapic acid ***	7.94	$C_{11}H_{11}O_5^-$	223.0612	223.0621	−0.90	**121.0306(100)**, 149.0245, 193.0153, 165.0183	**NF**	**NF**	+	**NF**

Table 2. *Cont.*

No.	Compound Names	t_R	Formula	Calculated Mass	*m/z* Exact Mass	Δ mDa	MS2 Fragments (%, Main Fragment)	Juice Samples			
								AJ	RB-AJ	BC-AJ	AM-AJ
27	**Sinapic acid hexoside**	6.53	$C_{17}H_{21}O_{10}^-$	385.1140	385.1150	−0.98	**205.0509(100)**, 206.0565, 164.0479, 179.0705, 149.0246, 223.0619	**NF**	**NF**	**+**	**NF**
28	**Disinapoyl–dihexoside**	8.48	$C_{34}H_{41}O_{19}^-$	753.2242	753.2218	2.40	**205.0508(100)**, 223.0611, 190.0278, 247.0619, 265.0726, 529.1577	**NF**	**NF**	**+**	**NF**
29	**Trisanapoyl–diglucoside**	9.02	$C_{45}H_{51}O_{23}^-$	959.2821	959.2887	−6.60	**735.2180(100)**, 205.0502, 247.0610, 265.0695, 511.1454, 529.159, 223.0615, 737.2217	**NF**	**NF**	**+**	**NF**
30	**Sinapoyl–malic acid**	7.94	$C_{15}H_{15}O_9^-$	339.0722	339.0753	−3.14	**149.0246(100)**, 164.0481, 179.0713, 193.0145, 208.0378, 223.0612, 133.0162, 115.0060	**NF**	**NF**	**+**	**NF**
					Flavonoids						
					Flavonols aglycones, glycosides, and acyl derivatives						
31	**Quercetin ***	9.37	$C_{15}H_9O_7^-$	301.0354	301.0357	−0.32	**151.0040(100)**, 107.0146, 121.0295, 178.9977, 229.0489, 245.0438, 273.0377	**+**	**+**	**+**	**+**
32	**Quercetin 3-*O*-rhamnoside**	8.22	$C_{21}H_{19}O_{11}^-$	447.0927	447.0945	−1.80	**300.0271(100)**, 301.0335, 271.0244, 255.0290, 151.0030, 178.9988, 121.0281, 341.0655	**+**	**+**	**+**	**+**
33	**Quercetin 3-*O*-hexoside**	7.77	$C_{21}H_{21}O_{12}^+$	465.1033	465.1048	−1.50	**303.0475(100)**	**+**	**+**	**+**	**+**

Table 2. *Cont.*

No.	Compound Names	t_R	Formula	Calculated Mass	*m/z* Exact Mass	Δ mDa	MS2 Fragments (%, Main Fragment)	Juice Samples			
								AJ	RB-AJ	BC-AJ	AM-AJ
34	**Patuletin**	9.37	$C_{16}H_{11}O_8^-$	331.0459	331.0482	−2.26	**165.9905(100)**, 121.0300, 181.0143, 193.9856, 243.0284, 271.0239, 287.0173, 316.0210	+	+	+	+
35	**Kaempferol 3-*O*-sinapoyl-dihexoside-7-*O*-hexoside**	6.80	$C_{44}H_{49}O_{25}^-$	977.2563	977.2593	−3.00	**815.2079(100)**, 609.1467, 591.1408, 446.0851, 429.0788, 284.0332	NF	NF	+	NF
36	**Kaempferol 3-*O*-sinapoyl-trihexoside-7-*O*-hexoside**	6.93	$C_{50}H_{59}O_{30}^-$	1139.3091	1139.3092	−0.10	**1139.3240(100)**, 977.2563, 815.2087, 771.1973, 609.2537, 591.1290, 429.0806, 284.0320, 205.0533	NF	NF	+	NF
					Flavan-3-ols and procyanidins						
37	**Catechin ***	6.03	$C_{15}H_{13}O_6^-$	289.0718	289.0719	−0.14	**123.0458(100)**, 109.0302, 121.0304, 125.0257, 137.0247, 151.0393, 159.0465 164.0153, 175.0476, 203.0700, 221.0806	+	+	+	+
38	**Epicatechin ***	6.80	$C_{15}H_{13}O_6^-$	289.0718	289.0723	−0.54	**123.0460(100)**, 109.0307, 121.0303, 125.0252, 137.0248, 149.0267, 151.0403, 161.05578, 187.0402, 203.0706, 221.0815	+	+	+	+

Table 2. *Cont.*

No.	Compound Names	t_R	Formula	Calculated Mass	*m/z* Exact Mass	Δ mDa	MS2 Fragments (%, Main Fragment)	Juice Samples			
								AJ	RB-AJ	BC-AJ	AM-AJ
39	**Procyanidin dimer B type is. I**	5.89	$C_{30}H_{25}O_{12}^-$	577.1352	577.1380	−2.85	**289.0718(100)**, 125.0253, 137.0244, 151.0394, 161.0251, 205.0423, 245.0762, 273.0394, 339.0861, 407.0776, 425.0868, 451.1058	+	+	+	+
40	**Procyanidin B2 *** (Procyanidin dimer B type is. II)	6.60	$C_{30}H_{25}O_{12}^-$	577.1352	577.1381	−2.95	**289.0718(100)**, 125.0257, 137.0248, 151.0402, 161.0256, 245.0753, 339.0858, 407.0789, 425.0884	+	+	+	+
41	**Chalcan–flavan 3-ol dimer**	7.68	$C_{27}H_{31}O_{14}^-$	579.1719	579.1766	−4.67	**245.0924(100)**, 125.0248, 151.0035, 167.0345, 203.0823, 271.0607, 289.0706, 409.0749	+	+	+	+
				Dihydrochalcones and glycosides							
42	**Phloretin ***	9.98	$C_{15}H_{13}O_5^-$	273.0768	273.0782	−1.35	**123.0456(100)**, 107.0173, 119.0490, 125.0277, 150.9984, 167.0341,	+	+	+	+
43	**Phloretin 2′-*O*-hexoside (Phlorizin)**	8.56	$C_{21}H_{23}O_{10}^-$	435.1291	435.1316	−2.50	**167.0355(100)**, 273.0762, 125.0256, 179.0344, 123.0453, 168.0379	+	+	+	+
44	**Phloretin 2′-*O*-(6′′-*O*-pentosyl)hexoside**	8.15	$C_{26}H_{31}O_{14}^-$	567.1714	567.1752	−3.80	**273.0788(100)**, 167.0353, 125.0250, 123.0461, 179.0347, 168.0377	+	+	+	+

Table 2. *Cont.*

No.	Compound Names	t_R	Formula	Calculated Mass	*m/z* Exact Mass	Δ mDa	MS2 Fragments (%, Main Fragment)	Juice Samples			
								AJ	RB-AJ	BC-AJ	AM-AJ
45	**3-Hydroxyphloretin**	9.28	$C_{15}H_{13}O_6^-$	289.0712	289.0725	−1.30	**123.0458(100)**, 167.0347, 125.025, 135.0448, 109.0317, 151.0035, 203.0724, 187.0438	+	**NF**	**NF**	**NF**
46	**3-Hydroxyphloretin-2′-*O*-hexoside**	8.08	$C_{21}H_{23}O_{11}^-$	451.1240	451.1265	−2.50	**167.0361(100)**, 125.0252, 289.0708, 123.0463, 271.0606, 179.0342	+	+	+	+
47	**3-Hydroxyphloretin-2′-*O*-(6″-*O*-pentosyl)hexoside**	7.85	$C_{26}H_{31}O_{15}^-$	583.1663	583.1700	−3.70	**289.0711(100)**, 167.0347, 125.0250, 271.0597, 168.0375, 123.0517	+	+	+	+
					Flavones						
48	**Chrysin** *	11.66	$C_{15}H_9O_4^-$	253.0501	253.0530	−2.90	**253.0497(100)**, 143.0510, 107.0162, 119.0513, 165.0687, 209.0586, 151.0044, 181.0639, 225.0525	+	**NF**	**NF**	**NF**
49	**Trihydroxyflavone (unknown)**	10.43	$C_{15}H_9O_5^-$	269.0450	269.0462	−1.20	**269.0453(100)**, 136.9884, 169.0656, 171.0445, 179.0495, 195.0448, 223.0392, 241.0492, 251.0359, 123.0092	+	+	+	+
					Apigenin C-glycosides						
					(*iso*)*vitexin derivatives*						

Table 2. *Cont.*

No.	Compound Names	t_R	Formula	Calculated Mass	*m/z* Exact Mass	Δ mDa	MS2 Fragments (%, Main Fragment)	Juice Samples			
								AJ	RB-AJ	BC-AJ	AM-AJ
50	**Isovitexin** *	7.68	$C_{21}H_{21}O_{10}^+$	433.1135	433.1147	−1.20	**313.0687(100)**, 283.0587, 323.0862, 297.0754, 337.0678, 343.0772, 351.0879, 379.0763, 397.0903, 415.1042	**NF**	**+**	**NF**	**NF**
51	**2″-Pentosyl (*iso*)vitexin**	7.61	$C_{26}H_{29}O_{14}^+$	565.1557	565.1566	−0.90	**313.0709(100)**, 283.0583, 433.1135, 415.1022, 397.0907, 379.0795, 367.0792, 351.0837, 343.0796, 337.0685	**NF**	**+**	**NF**	**NF**
52	**2″-Hexosyl (*iso*)vitexin**	7.54	$C_{27}H_{31}O_{15}^+$	595.1663	595.1669	−0.60	**313.0697(100)**, 283.0584, 295.0585, 433.1121, 415.1014, 397.0902, 379.0797, 367.0783, 337.0684	**NF**	**+**	**NF**	**NF**
53	**2″-Pentosyl-6″-malonyl (*iso*)vitexin**	8.09	$C_{29}H_{31}O_{17}^+$	651.1561	651.1573	−1.20	**313.0703(100)**, 271.0572, 283.0581, 295.0585, 607.1633, 519.1125, 501.1010, 483.0899, 475.1209, 457.1113, 439.1002, 337.0684	**NF**	**+**	**NF**	**NF**
54	**2″-Hexosyl-6″-malonyl (*iso*)vitexin**	7.98	$C_{30}H_{33}O_{18}^+$	681.1667	681.1681	−1.40	**313.0699(100)**, 295.0578, 283.0580, 271.0581, 519.1122, 501.1009, 483.0894, 475.1212, 457.1100, 439.1004, 433.1107, 415.0994, 397.0881, 379.0792, 337.0685	**NF**	**+**	**NF**	**NF**

Table 2. *Cont.*

No.	Compound Names	t_R	Formula	Calculated Mass	*m/z* Exact Mass	Δ mDa	MS2 Fragments (%, Main Fragment)	Juice Samples			
								AJ	RB-AJ	BC-AJ	AM-AJ
					Cytisoside derivatives						
55	**6″-Acetyl cytisoside**	9.30	$C_{24}H_{25}O_{11}^+$	489.1391	489.1405	−1.36	**327.0840(100)**, 351.0850, 309.0720, 297.0733, 369.0954, 429.1162, 471.1246, 447.1198, 411.1042	**NF**	**+**	**NF**	**NF**
56	**2″-Pentosyl cytisoside**	8.49	$C_{27}H_{31}O_{14}^+$	579.1714	579.1716	−0.20	**327.0866(100)**, 297.0740, 309.0744, 447.1293, 429.1180, 411.1066, 393.0953, 381.0945, 351.0849	**NF**	**+**	**NF**	**NF**
57	**2″-Rhamnosyl cytisoside**	8.39	$C_{28}H_{33}O_{14}^+$	593.1870	593.1895	−2.50	**447.1264(100)**, 429.1154, 411.1053, 393.0931, 381.0953, 351.0841, 327.0842, 309.0773, 297.0793	**NF**	**+**	**NF**	**NF**
58	**2″-Hexosyl cytisoside**	8.32	$C_{28}H_{33}O_{15}^+$	609.1819	609.1825	−0.60	**327.0861(100)**, 285.0784, 297.0742, 309.0740, 447.1287, 429.1173, 411.1061, 393.0947, 381.0948, 351.0842	**NF**	**+**	**NF**	**NF**
59	**2″-Hexosyl-6″-acetyl cytisoside**	8.63	$C_{30}H_{35}O_{16}^+$	651.1925	651.1926	−0.10	**489.1393(100)**, 471.1265, 429.1163, 411.1052, 381.0949, 369.0956, 351.0841, 327.0840, 309.0735, 297.0732, 285.0736	**NF**	**+**	**NF**	**NF**

Table 2. *Cont.*

No.	Compound Names	t_R	Formula	Calculated Mass	*m/z* Exact Mass	Δ mDa	MS2 Fragments (%, Main Fragment)	Juice Samples			
								AJ	RB-AJ	BC-AJ	AM-AJ
60	**2″-Hexuronyl-6″-acetyl cytisoside**	8.76	$C_{30}H_{33}O_{17}^+$	665.1718	665.1722	−0.40	**327.0853(100)**, 309.0737, 297.0737, 285.0729, 621.1798, 533.1274, 515.1159, 497.1053, 489.1372, 471.1260, 459.1256, 453.1161, 447.1274, 429.1155, 411.1043, 393.0949, 351.0842	**NF**	**+**	**NF**	**NF**
61	**2″-Hexosyl-6″-malonyl cytisoside**	8.63	$C_{31}H_{35}O_{18}^+$	695.1823	695.1836	−1.30	**327.0851(100)**, 309.0738, 297.0731, 285.0740, 533.1278, 515.1166, 497.1050, 489.1376, 471.1274, 453.1157, 447.1245, 429.1151, 411.1056, 393.0945, 375.0852, 351.0841	**NF**	**+**	**NF**	**NF**
					Betalamic acid and betalains						
62	**Betalamic acid**	3.77	$C_9H_{10}NO_5^+$	212.0559	212.0539	2.00	**120.0443(100)**, 130.0286, 148.0388, 166.0484	**NF**	**+**	**NF**	**+**
63	**Betanin**	5.08	$C_{24}H_{27}N_2O_{13}^+$	551.1513	551.1498	1.50	**389.0967(100)**, 150.0540, 297.0838, 343.0909, 345.1057, 551.1503,	**NF**	**+**	**NF**	**+**
64	**(*iso*)betanin**	3.30	$C_{24}H_{27}N_2O_{13}^+$	551.1513	551.1519	−0.60	**389.0962(100)**, 551.1481, 150.0539, 343.0903	**NF**	**+**	**NF**	**NF**

Table 2. *Cont.*

No.	Compound Names	t_R	Formula	Calculated Mass	*m/z* Exact Mass	Δ mDa	MS2 Fragments (%, Main Fragment)	Juice Samples			
								AJ	RB-AJ	BC-AJ	AM-AJ
65	**6′-*O*-Feruloylbetanin (Lampranthin II)**	7.41	$C_{34}H_{35}N_2O_{16}^+$	727.1987	727.1991	−0.40	**389.0976(100)**, 551.1516, 727.1974, 343.0943, 150.0536, 177.0533	**NF**	**+**	**NF**	**NF**
66	**2- or 17-Decarboxy(*iso*)betanin**	6.06	$C_{23}H_{27}N_2O_{11}^+$	507.1615	507.1604	1.10	**345.1059(100)**, 150.0535, 299.0993, 347.1113, 507.1575	**NF**	**+**	**NF**	**NF**
67	**2- or 17-Decarboxyneobetanin**	6.13	$C_{23}H_{25}N_2O_{11}^+$	505.1458	505.1453	0.50	**343.0899(100)**, 345.1067, 269.0894, 281.0887, 297.0851, 505.1434	**NF**	**+**	**NF**	**NF**
68	**Amaranthin**	2.68	$C_{30}H_{35}N_2O_{19}^+$	727.1834	727.1832	0.20	**389.0958(100)**, 551.1487, 727.1902, 150.0540, 343.0897, 297.0843	**NF**	**NF**	**NF**	**+**
69	**(*iso*)amaranhthin**	4.91	$C_{30}H_{35}N_2O_{19}^+$	727.1834	727.1853	−1.90	**389.0985(100)**, 551.1515, 727.1838, 150.0536, 343.0936, 297.0860	**NF**	**NF**	**NF**	**+**
70	**Decarboxy–dehydro–(*iso*)amaranthin**	5.86	$C_{29}H_{33}N_2O_{17}^+$	681.1779	681.1815	−3.60	**343.0913(100)**, 345.1070, 505.1447, 681.1771, 297.0847, 253.0951	**NF**	**NF**	**NF**	**+**
					Glucosinolate						
71	**Glucoraphanin ***	0.39	$C_{12}H_{22}NO_{10}S_3^-$	436.0406	436.0418	−1.20	**178.0157(100)**, 259.0105, 274.9857, 372.0383, 421.0141	**NF**	**NF**	**+**	**NF**

Abbreviations: **NF**, compound was not found/was not detected in juice sample; "**+**", compound found in analyzed juice samples. Samples: "**AJ**", apple juice (control juice); "**RB-AJ**", red beet microgreens–apple juice; "**BC-AJ**", broccoli–apple juice; "**AM-AJ**", amaranth microgreens–apple juice. * Compound confirmed by comparison with available standards.

Among the detected compounds (Table 2), several phenolic compounds (**5**, **21**, **26**, **31**, **37**, **38**, **40**, **42**, **48**, and **50**) and glucoraphanin (**71**) were authenticated by direct comparison with reference standards (#Category 1—verified compounds) and are marked in Table 2 (see *). Other reported compounds were tentatively identified based on their exact m/z values (monoisotopic mass), characteristic MS fragmentation patterns, and typical MS/MS fragments (#Category 2—putatively identified compounds). The mean mass accuracy for these annotations was <5 mDa, for both precursor ions and major fragments, indicating high identification confidence.

Phenolic acid derivatives were the most frequently identified compounds, which is expected given their wide occurrence in apples [31,42,43] and red beet, broccoli, or amaranth microgreens [3,6]. Free phenolic acids were rarely detected; only hydroxybenzoic acid (**1**), protocatechuic acid (**5**), and sinapic acid (**26**) were identified and confirmed by comparison with the available standards (**2** and **26**). Hydroxybenzoic acids were mainly detected in the form of monoglycosides (pentoside or hexoside) and dyglycosides. All monoglycosides (**2**, **3**, **6**, **7**, **9**, **12**, and **13**) showed unique monoisotopic mass and characteristic fragment (deprotonated phenolic acids) obtained by the loss of a pentosyl (−132 Da) or hexosyl (−162 Da) unit, as well as accompanying fragments resulting from cleavage of the phenolic aglycone (i.e., neutral losses of CH_3 (−15 Da) and/or CO_2 (−44 Da)). Compounds **2** and **3** were isomers of hydroxybenzoic acid hexosides, showing identical precursor masses and MS/MS fragments (137 m/z), but different retention times. Identified monohexosides of hydroxybenzoic acids have been previously reported in various apple cultivars [31,42], indicating that their presence in microgreen–apple juices likely originates from the apple rather than microgreens. Compounds **4** and **10** were recognized as pentosyl–hexosides of hydroxybenzoic acid and vanillic acid, respectively, showing typical fragments formed by the sequential loss of pentosyl (+H_2O) and hexosyl units to deprotonated aglycones. Compound **8** was identified as acetyl–vanillic acid (m/z 209), showing characteristic fragments resulting from the loss of the acetyl group (−42 Da) and subsequent cleavage of the vanillic acid moiety. Vanilloside or Glucovanillin (**11**) were identified based on characteristic vanillin-related fragments (151 m/z, 135 m/z, 123 m/z, and 107 m/z). Compound **14** was identified as shikimoylquinic acid (m/z 527), showing fragments at 353 m/z (the loss of the shikimoyl moiety) and 191 m/z (deprotonated quinic acid) → 173 m/z (-H_2O). Among the reported compounds, dihydroxybenzoic acid pentoside (**6**) was exclusively detected in red beet–apple juice, phenolic acid diglycosides (**4** and **10**) in amaranth–apple juice, and acetyl–vanillic acid (**8**) in broccoli–apple juice, indicating their origin from the respective microgreens. On the other hand, all identified hydroxycinnamic acid derivatives corresponded to coumaric, caffeic, ferulic, and sinapic acid derivatives. The aforementioned hydroxycinnamic acids most commonly occurred as esters (conjugates) with quinic (compounds **16**, **17**, and **20**–**23**), (*iso*)citric (**18**, **23**, and **25**), or malic acid (**30**), and were detected in various isomeric forms. Cinnamate esters with quinic and (*iso*)citric acids exhibited similar MS fragmentation patterns, but distinct monoisotopic masses, molecular formulas, and specific fragments corresponding to deprotonated quinic acid [**191.055** m/z → 173.045 m/z (−H_2O) → 155.035 m/z (−H_2O) → 127.041 m/z (−CO)] and deprotonated (*iso*)citric acid [**191.019** m/z → 173.010 m/z (−H_2O) → 154.999 m/z (−H_2O) → 111.010 m/z (−CO_2)]. The identification of these compounds is further supported by the fragmentation pathways of compounds **16** (Figure 1a) and **18** (Figure 1b). Compound **23** was identified as methyl–caffeoylquinate (rather than feruloylquinic acid), based on fragment ions at 179 m/z, 161 m/z (-H_2O), and 135 m/z (-CO_2), related to the deprotonated caffeoyl moiety. Cinnamate esters with quinic acid, including methyl–caffeoylquinate, have been previously reported in apple fruit (mesocarp and peel) [27,42,43], juices [29], and cider [29,44]. In contrast, coumaroyl–(*iso*)citric acid (m/z 337.0560), caffeoyl–(*iso*)citric acid

(*m*/*z* 353.0509), and feruloyl–(*iso*)citric acid (*m*/*z* 367.0671) were detected only in amaranth–apple juice, representing compounds exclusively derived from amaranth microgreens. These hydroxycinamoyl–(*iso*)citric acid derivatives have been previously identified and reported in *Amaranthus cruentus* [45] and *Amaranthus viridis* [46].

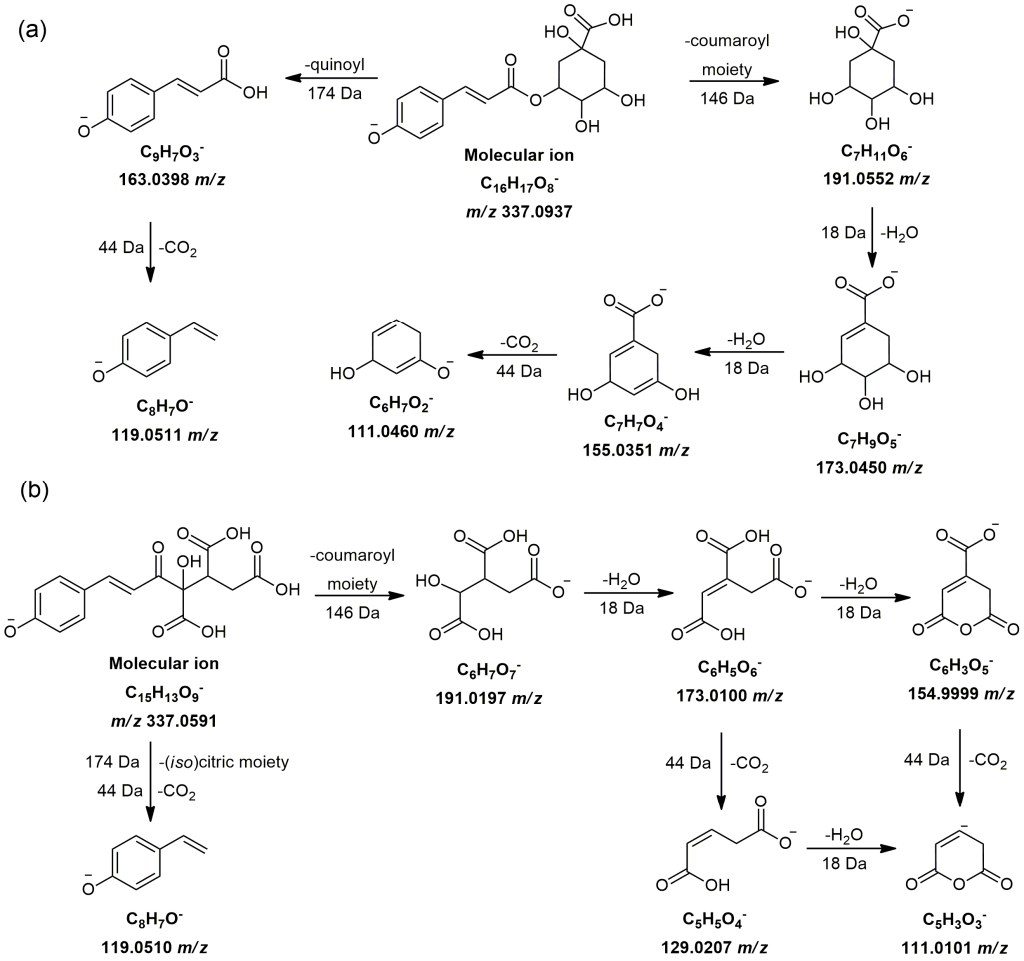


Figure 1. Proposed fragmentation pathways of (**a**) coumaroylquinic acid is. I (**16**) and (**b**) coumaroyl–(*iso*)citric acid (**18**).

Compound **30** was recognized as sinapoyl–malic acid, showing characteristic fragments corresponding to deprotonated sinapic acid [223 *m*/*z*, 208 *m*/*z* (-CH_3), 193 *m*/*z* (−2 × CH_3), 179 *m*/*z* (-CO_2), 164 *m*/*z* (-CH_3, -CO_2), 149 *m*/*z* (−2 × CH_3, -CO_2)], and deprotonated malic acid [133 *m*/*z* → 115 *m*/*z* (-H_2O)]. Coumaric, caffeic, and sinapic acid monohexosides (compounds **15**, **19**, and **27**) showed typical fragments resulting from the loss of a hexosyl unit (−162 Da), loss of methyl group(s) (−15 Da), in the case of sinapic acid, and cleavage of deprotonated phenolic acids (neutral losses of CO_2 and H_2O). Except for sinapic acid monohexoside, two complex sinapoyl–hexoside derivatives (**28** and **29**) were identified and recognized as disinapoyl–dihexoside (*m*/*z* 753) and trisinapoyl–dihexoside (*m*/*z* 959). These sinapoyl–hexoside derivatives showed typical MS fragmentation patterns, with characteristic fragments obtained by sequential losses of hexosyl units and sinapoyl moieties. Sinapic acid and identified sinapoyl derivatives (**27**–**30**) were detected only in the broccoli–apple juice and represent typical compounds derived from broccoli microgreens. These sinapoyl compounds have been previously reported in broccoli microgreens [3,6] and other *Brassica* species [47–49].

Alongside phenolic acid derivatives, various flavonoids have been identified in the analyzed juices and can be classified into several groups: **(a)** flavonol aglycones, glycosides,

and acyl derivatives (six compounds); **(b)** flavan-3-ols and procyanidins (five compounds); **(c)** dihydrochalcones and their glycosides (six compounds); **(d)** flavones (two compounds); and **(e)** apigenin *C*-glycosides (12 compounds). Except for apigenin *C*-glycosides in red beet and certain quercetin and kaempferol derivatives in broccoli [3,6], the analyzed microgreens are generally low in flavonoid content. Therefore, most of the identified flavonoids (flavonols, flavan-3-ols, procyanidins, and dihydrochalcones) likely originate from the apples [27,29,31,43,50] and are transferred into the juice during the squeezing process. Flavonol (quercetin, **31**), flavan-3-ol (catechin, **37**; and epicatechin, **38**), and flavone (chrysin, **48**) aglycones were established based on characteristic fragments generated by Retro-Diels–Alder (RDA) ($^{0,4}A^-$; $^{1,4}A^-$; $^{1,2}A^-$; $^{1,2}B^-$; $^{1,3}A^-$; and/or $^{1,3}B^-$) cleavage and neutral losses (CO, CO_2, H_2O, C_2H_2O, and C_3O_2), and subsequently confirmed by comparison with authentic standards. Catechin (**37**) and epicatechin (**38**) showed the same m/z exact mass (m/z 289), formulas ($C_{15}H_{14}O_6$), MS fragmentation patterns, and main fragments [123 m/z ($^{1,2}B^-$) →109 m/z (-CH_2), 125 m/z ($^{1,4}A^-$), 137 m/z ($^{1,3}A^-$), 151 m/z ($^{1,3}B^-$), 203 m/z (-H_2O,-C_3O_2; or -CO_2, -C_2H_2O)], but different elution time. Compound **34** was tentatively identified as patuletin based on its characteristic fragments at 181 m/z ($^{1,3}A^-$) → 166 (165.99 m/z) (-CH_3), 121 m/z ($^{1,2}B^-$), 194 (193.98 m/z) ($^{1,2}A^-$; -CH_3), [316 m/z (-CH_3) → 287 m/z (-H, -CO) → 271 m/z (O) → 243 m/z (-CO)]. Patuletin and its glycosides have previously been identified and reported in thinned young apple fruits [51]. Compound **49** remains unidentified; however, based on its monoisotopic mass (m/z 269), predicted molecular formula ($C_{15}H_{10}O_5$), and MS/MS fragmentation pattern, it is tentatively characterized as a trihydroxyflavone. In addition to the aglycone, glycosides of quercetin were also identified. Quercetin 3-*O*-rhamnoside (**32**) was detected in negative ionization mode, exhibiting major fragments at 301/300 m/z (Y_0^-/[Y_0–H]$^-$), along with characteristic RDA fragments of quercetin (151 m/z, 179 m/z, and 121 m/z). On the other hand, quercetin 3-*O*-hexoside (m/z 465) (**33**) was detected in positive ionization mode, showing a characteristic fragment at 303 m/z (protonated quercetin), resulting from the loss of a hexosyl unit. Compounds **35** and **36** were tentatively identified as complex kaempferol sinapoyl polyglycosides, exhibiting similar MS fragmentation patterns, with major fragments formed through sequential losses of hexosyl units (+H_2O) and a sinapoyl moiety. These compounds have previously been identified in broccoli microgreens [3,6] and other *Brassica* species [47,52], which explains their exclusive presence in the broccoli–apple juice. Compounds **39**–**41** belong to polymerized flavan-3-ols, i.e., isomeric procyanidin dimers and a chalcan–flavan-3-ol dimer. Procyanidin B2 (**40**) was additionally confirmed using an authentic standard. These polymeric compounds showed characteristic fragments at 451 m/z, 425 m/z, 407 m/z (409 m/z), and 289 m/z, resulting from heterocyclic ring fission (the loss of a phloroglucinol unit, −126 Da), retro-Diels–Alder cleavage (elimination of a hydroxyvinyl benzenediol unit, −152 Da), and/or quinone methide cleavage (fragmentation of the interflavanic bond between two monomer units) [53,54].

Dihydrochalcones (especially phloretin and its 2′-*O*-glycosides) are characteristic apple bioactive compounds predominantly concentrated in the peel [31,42,43], which are transferred into the juice during the squeezing process [29,55]. As previously reported, these compounds exhibit multiple pharmacological effects, including antidiabetic, anti-inflammatory, antihyperglycemic, anticancer, and antibacterial activities [43,56,57]. Phloretin (**42**) and 3-hydroxyphloretin (**45**) were identified based on characteristic fragments: **(a)** 167 m/z (A-ring) and [119 m/z (**42**) or 135 m/z (**45**)] (B-ring) formed by cleavage of the bond between the carbonyl carbon (C=O) and α-carbon (Retro-Michael addition via heterolytic cleavage); (b) 167 m/z (A-ring) → 125 m/z (loss of a ketene molecules, −42 Da); and (c) 167 m/z → 123 m/z (rearrangement followed by loss of CO_2). 3-hydroxyphloretin (**45**) exhibited the same monoisotopic mass (m/z 289) and molecular formula ($C_{15}H_{14}O_6$)

as catechin and epicatechin, but differed in its fragmentation pattern and retention time. In addition to the aglycones, phloretin and 3-hydroxyphloretin were also identified in the forms of 2′-*O*-hexoside (**43** and **46**) and 2′-*O*-(6″-*O*-pentosyl)hexoside (**44** and **47**). The key fragments for the identification of these glycosides were 273 *m*/*z* (deprotonated phloretin; **43** and **44**) and [289 *m*/*z* (deprotonated 3-hydroxyphloretin) → 271 *m*/*z* (-H_2O, −18 Da)] (**46** and **47**), followed by fragments at 167 *m*/*z*, 125 *m*/*z* and 123 *m*/*z*, formed by cleavage of deprotonated aglycones. Fragments at 273 *m*/*z* and 289 *m*/*z* were obtained by loss of the hexosyl unit (−162 Da) and/or a 6″-*O*-pentosylhexoside moiety (−294 Da).

Apigenin *C*-glycosides, including isovitexin (**50**), as well as various (*iso*)vitexin and cytisoside (3′-O-methylvitexin) glycosides and acyl derivatives (**51**–**61**), were detected exclusively in the red beet–apple juice. These compounds exhibit characteristic MS/MS fragmentation patterns in positive ionization mode, providing typical fragments useful for their structural elucidation. Isovitexin (*m*/*z* 433; **50**) was identified based on characteristic fragments at 415 *m*/*z* (−H_2O), 397 *m*/*z* (−2 × H_2O), 343 *m*/*z* (−3 × CH_2O or $^{0,3}X^+$ scission), 337 *m*/*z* (−2 × H_2O, −2 × CH_2O), and 313 *m*/*z* (−4 × CH_2O or $^{0,2}X^+$ scission), and was confirmed by alignment with an authentic reference standard. (*iso*)vitexin glycosides showed a characteristic fragment at 433 *m*/*z*, obtained by loss of a pentosyl (**51**) or a hexosyl (**52**) unit. Compounds **53** and **54** were tentatively identified as 2″-*O*-pentosyl-6″-*O*-malonyl (*iso*)vitexin (*m*/*z* 651) and 2″-*O*-hexosyl-6″-*O*-malonyl (*iso*)vitexin (*m*/*z* 681), respectively, exhibiting major fragments at 519 *m*/*z* and 433 *m*/*z*, resulting from sequential losses of a pentosyl or hexosyl unit and a malonyl moiety. All cytisoside glycosides and their acyl derivatives exhibited the same MS base fragments at 447 *m*/*z* (protonated cytisoside), 429 *m*/*z* (−H_2O), 411 *m*/*z* (−2 × H_2O), 351 *m*/*z* (−2 × CH_2O, −2 × H_2O), 327 *m*/*z* (−4 × CH_2O or $^{0,2}X^+$ scisssion), 309 *m*/*z* ($^{0,2}X^+$ scission, −H_2O), and 297 *m*/*z* ($^{0,2}X^+$ scission, −CH_2O). Compounds **55**, **56**, **57**, and **58** were identified as 6″-*O*-acetyl cytisoside (*m*/*z* 489), 2″-*O*-pentosyl cytisoside (*m*/*z* 579), 2″-*O*-rhamnosyl cytisoside (*m*/*z* 593), and 2″-*O*-hexosyl cytisoside (*m*/*z* 609), respectively, all showing a fragment at 447 *m*/*z*, resulting from the loss of *O*-bound substituents acetyl (−42 Da), pentosyl (−132 Da), rhamnosyl (−146 Da), and hexosyl (−162 Da), respectively. Compounds **59**–**61** were identified as acyl derivatives of cytisoside, exhibiting fragments obtained by sequential losses of a hexosyl or hexuronyl unit and an acetyl or malonyl moiety. Moreover, the fragmentation pathway of 2″-Hexuronyl-6″-acetyl cytisoside (**60**) was previously reported and described in detail [3]. Some of the identified (*iso*)vitexin and cytisoside derivatives have been previously reported in *Beta vulgaris* cell cultures, microgreens, stalks, and leaves [3,58–60], and characterized by NMR spectroscopy [58], with documented antioxidant, anticancer, and anti-inflammatory activities [59,61]. Their presence further enhances the nutritional value and antioxidant potential of the analyzed red beet–apple juice.

Besides phenolic compounds, several characteristic betalains and one glucosinolate were identified and selectively found in the analyzed microgreen–apple juice samples. As shown in Table 2, various betalains were detected only in the red beet–apple and/or amaranth–apple juices, which is consistent with their high water solubility and presence in these microgreens. Betalamic acid (**62**) was detected in both juices, as expected, since it represents a key structural component and biosynthetic precursor of all betalains. Compounds **63** and **64** were identified as betanin and (*iso*)betanin, showing the same exact mass (*m*/*z* 551) and similar MS fragmentation patterns, but different retention times. Compounds **68** and **69** were detected only in amaranth–apple juice and were identified as amaranthin and (*iso*)amaranthin (*m*/*z* 727), respectively. The reported compounds exhibited the same main fragments at 389 *m*/*z*, 343 *m*/*z*, and 150 *m*/*z*, corresponding to the protonated betanidin aglycone and its characteristic cleavage products [62]. The key fragment at 389 *m*/*z* was formed via loss of a glucosyl–glucuronyl residue for (*iso*)amaranthin or a glucosyl unit for

(*iso*)betanin. Compound **65** was identified as 6′-*O*-feruloylbetanin (Lampranthin II), exhibiting similar MS fragmentation patterns and fragments to amaranthin/(*iso*)amaranthin, but a difference in molecular formula ($C_{34}H_{34}N_2O_{16}$) and monoisotopic mass (*m*/*z* 727.1991) (Figure 2a,b). Moreover, 6′-*O*-feruloylbetanin was detected only in red beet–apple juice. Compounds **66**, **67**, and **70** were identified as decarboxylated (-CO_2) forms of (*iso*)betanin, neobetanin, and (*iso*)amaranthin, respectively, likely formed in juice under acidic conditions and/or upon exposure to oxygen during cold pressing of red beet and amaranth microgreens. The decarboxylation process is often accompanied by dehydrogenation, as in the case of detected decarboxy–dehydro–(*iso*)amaranthin (**70**).

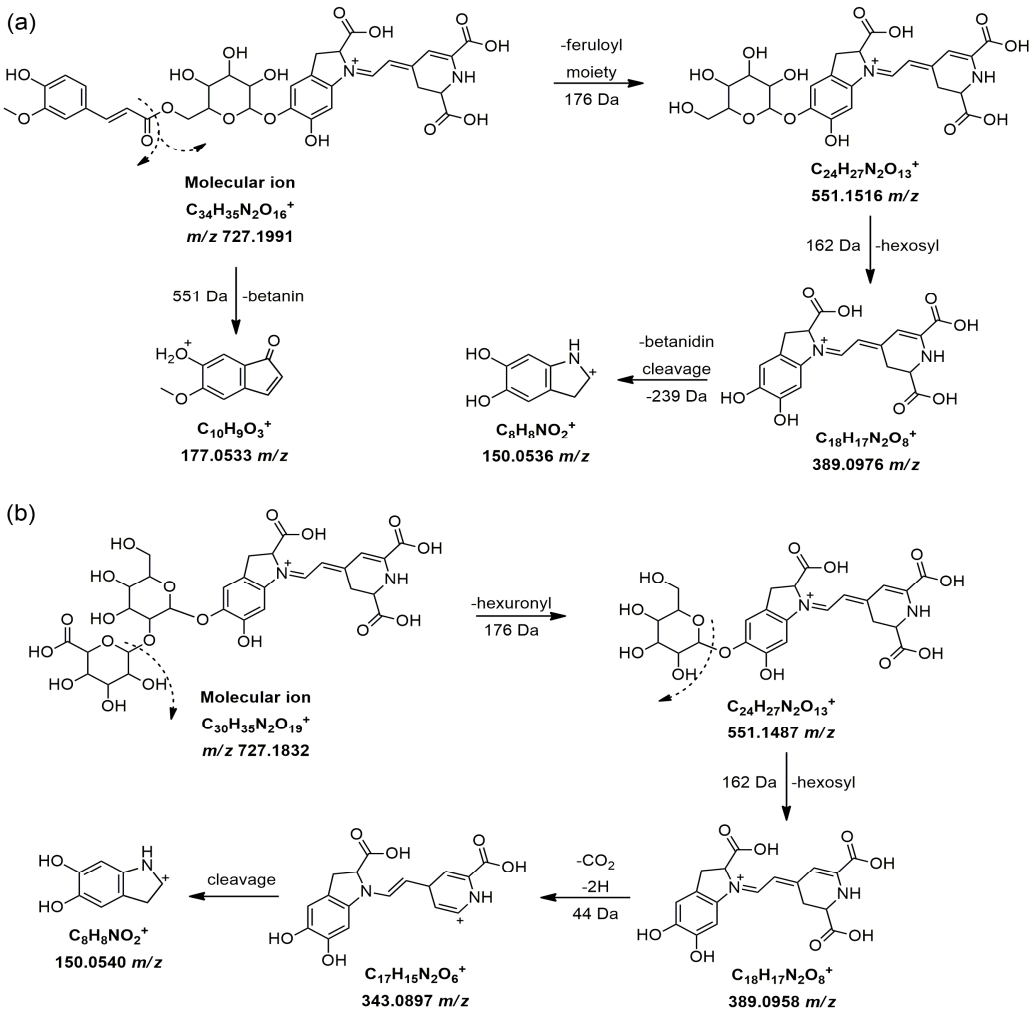


Figure 2. Proposed fragmentation pathway of (**a**) 6-*O*-feruloylbetanin (lampranthin II) (**65**) and (**b**) amaranthin (**68**).

Glucosinolates are major bioactive compounds in broccoli microgreens [3,63,64]; however, in the broccoli–apple juice, only glucoraphanin (**71**) was detected and confirmed by comparison with an authentic standard. However, the results from quantitative analysis showed that glucoraphanin was present only in trace amounts (<LOD). The low level of glucoraphanin and the absence of other broccoli glucosinolates may be attributed to their hydrolysis to isothiocyanates due to myrosinase activity [65]. Notably, sulforaphane, the principal isothiocyanate derived from glucoraphanin, was not detected in the broccoli–apple juice. This may be explained by its instability and subsequent degradation to sulforaphane–amine or other cleavage products, as well as its ability to form conjugates with glutathione or plant proteins present in the juice [15]. Nevertheless, isothiocyanate degradation prod-

ucts and conjugated forms also exhibit significant biological activity [12,13,15], and potentially contribute to the antioxidant potential of broccoli–apple juice.

To visualize relationships within the metabolomic datasets, identify discriminant compounds, provide a more rigorous comparison among juices, and confirm previous observations, the metabolomic data (peak areas) were analyzed using multivariate unsupervised statistical methods, namely principal component analysis (PCA) and hierarchical cluster analysis (HCA) (Figure 3).

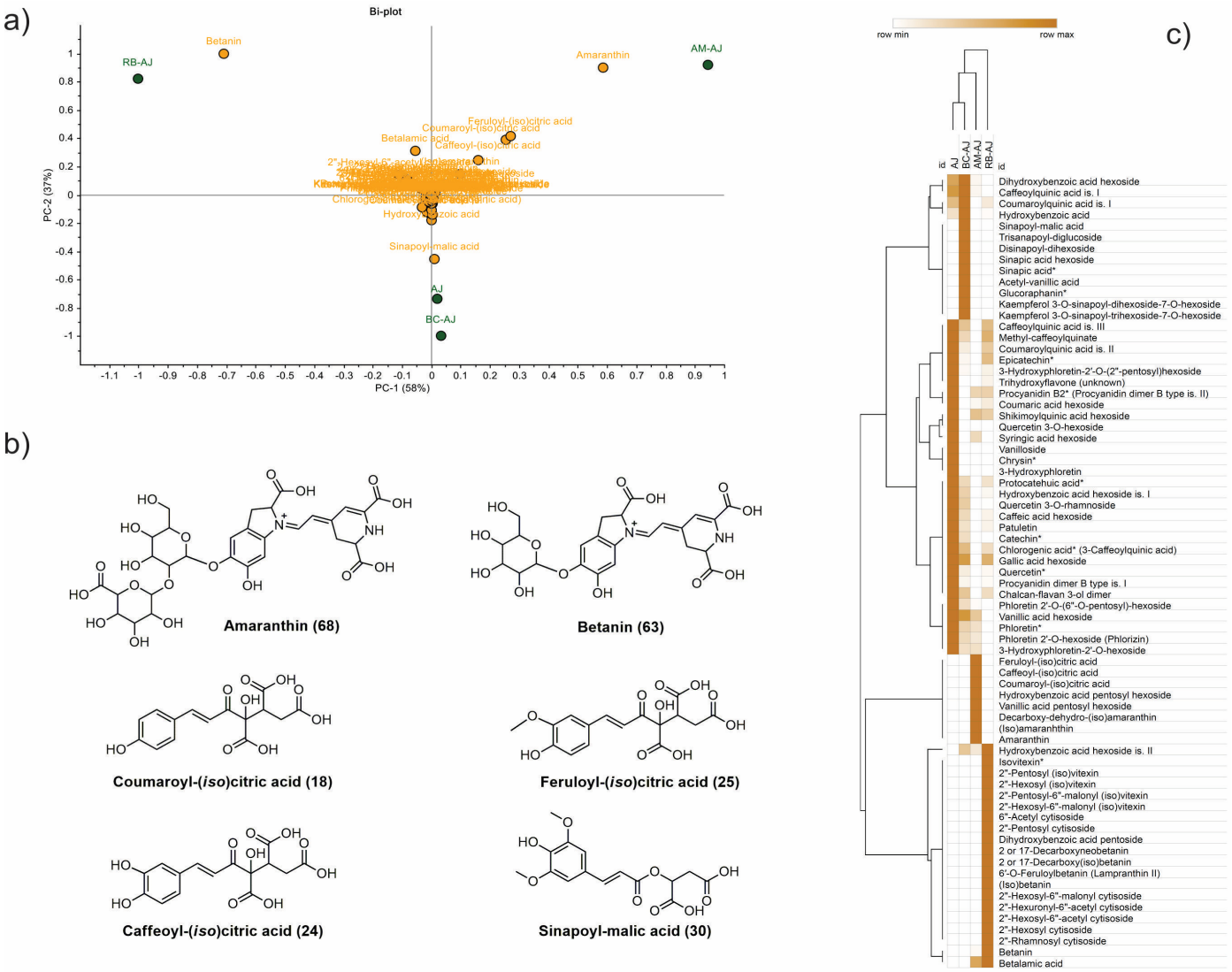


Figure 3. (**a**) A principal component analysis (PCA) biplot generated based on the QToF MS data, with the first two PCs (PC1 and PC2) explaining 58% and 37% of the variability, respectively; (**b**) the structures of the main discriminant metabolites contributing to the separation of microgreen–apple juices in the PCA—biplot; and (**c**) a heatmap of the scaled QToF MS data with the samples and compounds arranged according to the hierarchical cluster analysis, using Spearman's method of cluster agglomeration; "*" compounds confirmed by the available standards.

As shown, the first two principal components (PC1 and PC2) explained 58% and 37% of the total variability, respectively (Figure 3a). PCA grouped RB-AJ and AM-AJ separately from the other juices (AJ and BC-AJ) along PC2 (Figure 3a), due to betanin and amaranthin, which are predominantly concentrated in red beet and amaranth microgreens and juices. When considering both principal components together (PC1 and PC2), a clear separation among all microgreen–apple juices was observed (Figure 3a), due to the characteristic and

most abundant compounds derived from amaranth, red beet, and broccoli microgreens. The main discriminant metabolites contributing to the separation of microgreen–apple juices in the PCA biplot were betanin (RB-AJ), sinapoyl–malic acid (BC-AJ), and amaranthin, together with hydroxycinnamoyl–isocitric acid derivatives (AM-AJ) (Figure 3b).

The HCA subclusters were consistent with the PCA-based separation (Figure 3c). All the juices belonged to the same main cluster due to the presence of phenolic compounds originating from apples, which were distributed across all the microgreen–apple juices. However, three distinct subclusters corresponding to the individual microgreen–apple juices were observed (Figure 3c), due to the presence of phytochemical markers (compounds) specific to each microgreen species: sinapic acid and sinapoyl derivatives for the BC-AJ; amaranthin, hydroxycinnamoyl–isocitric acid derivatives, and phenolic acid diglycosides for the AM-AJ; and vitexin derivatives, cytisoside derivatives, betanin, and its derivatives for the RB-AJ.

3.3. Content of Identified Phenolic Compounds in Cold-Pressed Microgreen–Apple Juices

As shown in Table 2, phenolic compounds were the most abundant compounds identified in the analyzed juices. For these reasons, they were quantified or semi-quantified to determine their individual and total contents, as well as their distribution in the apple and microgreen–apple juices (Table 3).

Table 3. Content (μg/100 mL of juices) of (tentatively) identified phenolic compounds in apple and microgreen–apple juices (RB-AJ, BC-AJ, and AM-AJ), using UHPLC Q-ToF MS.

No.	Compound Name	Juices (μg/100 mL)			
		AJ	RB-AJ	BC-AJ	AM-AJ
	Phenolic acids				
	Hydroxybenzoic acids and derivatives **(*a*)**				
1	Hydroxybenzoic acid	28.37	11.47	99.62	8.78
2	Hydroxybenzoic acid hexoside is. I	33.25	11.73	18.19	10.29
3	Hydroxybenzoic acid hexoside is. II	—	7.31	2.63	0.71
4	Hydroxybenzoic acid pentosyl hexoside	—	—	—	37.15
5	Protocatehuic acid *	9.41	2.99	3.55	<LOQ
6	Dihydroxybenzoic acid pentoside	—	41.39	—	—
7	Dihydroxybenzoic acid hexoside	20.03	3.49	28.61	5.22
8	Acetyl–vanillic acid	—	—	6.15	—
9	Vanillic acid hexoside	13.04	5.98	11.35	8.85
10	Vanillic acid pentosyl hexoside	—	—	—	45.11
11	Vanilloside	11.34	—	—	—
12	Gallic acid hexoside	39.25	29.04	32.52	14.86
13	Syringic acid hexoside	9.72	—	—	2.24
14	Shikimoylquinic acid hexoside	3.78	<LOQ	—	1.35
	Hydroxycinnamic acids and derivatives				
	Coumaric acid derivatives **(*b*)**				
15	Coumaric acid hexoside	6.12	3.23	2.76	2.96
16	Coumaroylquinic acid is. I	8.74	3.70	16.06	1.43
17	Coumaroylquinic acid is. II	8.05	4.86	3.50	2.79

Table 3. *Cont.*

No.	Compound Name	Juices (µg/100 mL)			
		AJ	RB-AJ	BC-AJ	AM-AJ
18	Coumaroyl–(*iso*)citric acid	—	—	—	59.82
	Caffeic acid derivatives **(*c*)**				
19	Caffeic acid hexoside	6.68	<LOQ	<LOQ	<LOD
20	Caffeoylquinic acid is. I	<LOQ	<LOD	<LOQ	<LOD
21	Chlorogenic acid * (3-Caffeoylquinic acid)	106.76	65.68	78.91	51.49
22	Caffeoylquinic acid is. III	12.81	6.03	5.29	—
23	Methyl–caffeoylquinate	5.78	<LOQ	<LOQ	—
24	Caffeoyl–(*iso*)citric acid	—	—	—	31.18
	Ferulic acid derivatives **(*d*)**				
25	Feruloyl–(*iso*)citric acid	—	—	—	700.24
	Sinapic acid and derivatives **(*e*)**				
26	Sinapic acid *	—	—	212.33	—
27	Sinapic acid hexoside	—	—	158.98	—
28	Disinapoyl–dihexoside	—	—	24.59	—
29	Trisanapoyl–diglucoside	—	—	53.79	—
30	Sinapoyl–malic acid	—	—	677.96	—
	Flavonoids				
	Flavonols aglycones, glycosides, and acyl derivatives **(*f*)**				
31	Quercetin *	2.63	<LOD	<LOQ	<LOD
32	Quercetin 3-*O*-rhamnoside	2.22	<LOQ	1.10	<LOQ
34	Patuletin	1.12	<LOD	<LOD	<LOD
35	Kaempferol 3-*O*-sinapoyl-dihexoside-7-*O*-hexoside	—	—	1.26	—
36	Kaempferol 3-*O*-sinapoyl-trihexoside-7-*O*-hexoside	—	—	0.67	—
	Flavan-3-ols and procyanidins **(*g*)**				
37	Catechin *	37.99	13.75	19.70	13.68
38	Epicatechin *	32.11	21.85	13.23	11.92
39	Procyanidin dimer B type is. I	74.05	20.62	23.22	18.79
40	Procyanidin B2 * (procyanidin dimer B type is. II)	58.14	35.20	23.19	34.26
41	Chalcan–flavan 3-ol dimer	38.64	10.98	17.39	<LOQ
	Dihydrochalcones and glycosides **(*h*)**				
42	Phloretin *	0.62	<LOQ	<LOQ	<LOQ
43	Phloretin-2′-*O*-hexoside (Phlorizin)	3.93	0.96	1.77	1.46
44	Phloretin 2′-*O*-(6″-*O*-pentosyl)hexoside	4.70	1.86	2.57	1.95
45	3-Hydroxyphloretin	0.36	—	—	—
46	3-Hydroxyphloretin-2′-*O*-hexoside	4.05	1.53	2.12	2.11
47	3-Hydroxyphloretin-2′-*O*-(2″-*O*-pentosyl)hexoside	2.94	1.34	1.24	1.15

Table 3. *Cont.*

No.	Compound Name	Juices (µg/100 mL)			
		AJ	**RB-AJ**	**BC-AJ**	**AM-AJ**
	Flavones **(*i*)**				
48	Chrysin *	<LOD	—	—	—
49	Trihydroxyflavone (unknown)	1.14	<LOD	<LOD	<LOD
	Apigenin C-glycosides **(*j*)**				
50	Isovitexin *	—	3.09	—	—
51	2″-Pentosyl (*iso*)vitexin	—	34.75	—	—
52	2″-Hexosyl (*iso*)vitexin	—	24.68	—	—
53	2″-Pentosyl-6″-malonyl (*iso*)vitexin	—	35.24	—	—
54	2″-Hexosyl-6″-malonyl (*iso*)vitexin	—	31.78	—	—
55	6″-Acetyl cytisoside	—	3.94	—	—
56	2″-Pentosyl cytisoside	—	57.83	—	—
57	2″-Rhamnosyl cytisoside	—	4.61	—	—
58	2″-Hexosyl cytisoside	—	49.78	—	—
59	2″-Hexosyl-6″-acetyl cytisoside	—	71.42	—	—
60	2″-Hexuronyl-6″-acetyl cytisoside	—	19.74	—	—
61	2″-Hexosyl-6″-malonyl cytisoside	—	22.86	—	—
	∑∑	**587.78**	**664.74**	**1544.25**	**1069.77**

Abbreviations: "**—**", compound was not found in juice sample. Samples: "**AJ**", apple juice (control juice); "**RB-AJ**", red beet microgreens–apple juice; "**BC-AJ**", broccoli microgreens–apple juice; "**AM-AJ**", amaranth microgreens–apple juice. * Compound quantified using corresponding standards: **(*a*)** expressed as **protocatehuic acid** equivalents (µg PA/100 mL); **(*b*)** expressed as **coumaric acid** equivalents (µg CoA/100 mL); **(*c*)** expressed as **caffeic acid** equivalents (µg CaA/100 mL); **(*d*)** expressed as **ferulic acid** equivalents (µg FA/100 mL); **(*e*)** expressed as **sinapic acid** equivalents (µg SA/100 mL); **(*f*)** expressed as **quercetin** equivalents (µg QE/100 mL); **(*g*)** expressed as **procyanidin B2** equivalents (µg PB2/100 mL); **(*h*)** expressed as **phloretin** equivalents (µg PH/100 mL); **(*i*)** expressed as **chrysin** equivalents (µg CH/100 mL); and, **(*j*)** expressed as **isovitexin** equivalents (µg IsV/100 mL).

The highest total content of all identified phenolics was found in broccoli–apple juice (1544.25 µg/100 mL), followed by amaranth–apple juice (1069.77 µg/100 mL) and red beet–apple juice (664.74 µg/100 mL). The total phenolic content in the control apple juice was significantly lower than in the microgreen–apple juices and in the apple juices reported in the literature [29–31]. Phenolic compounds derived from the microgreens mainly contributed to the differences in total phenolic content among microgreen–apple juices, given that the distribution of individual phenolics originating from apples was similar across these juices. Phenolic acids and their derivatives were the predominantly quantified phenolic compounds in all of the analyzed juices, due to their widespread presence in both apples and microgreens [3,27,66], as well as their high ability to migrate into juices [31]. Among hydroxybenzoic acids, dihydroxybenzoic acid pentoside (41.39 µg PA/100 mL) was predominantly and exclusively quantified in the red beet–apple juice, while hydroxybenzoic acid pentosyl hexoside (37.15 µg PA/100 mL) and vanillic acid pentosyl hexoside (45.11 µg PA/100 mL) were quantified in the amaranth–apple juice. In the broccoli–apple juice, high contents of hydroxybenzoic acid, dihydroxybenzoic acid hexoside, and gallic acid hexosides were found, originating from both the apples and broccoli microgreens. Hydroxycinnamic acid derivatives were predominantly present in the broccoli–apple and amaranth–apple juices, mainly contributed by sinapic acid derivatives (sinapic acid, 212.33 µg/100 mL; sinapoyl–malic acid 677.96 µgSA/100 mL; 158.98 µgSA/100 mL;

and trisinapoyl–diglucoside, 53.79 µgSA/100 mL), and hydroxycinnamoyl–isocitric acid compounds (feruloyl–(*iso*)citric acid, 700.24 µgFA/100 mL; coumaroyl–(*iso*)citric acid, 59.82 µgCA/100 mL; and caffeoyl–(*iso*)citric acid, 31.18 µgCA/100 mL), respectively. In all microgreen–apple juices, a high content of chlorogenic acid was observed (51.49–78.91 µg/100 mL), primarily originating from the apple juice and its presence in juice blends. Other studies have also reported a high content of chlorogenic acid in both apple fruits [27,28] and apple juice [30,31,67,68].

Quercetin, its rhamnoside, and patuletin were quantified only in the apple juice at low concentrations (1.12–2.63 µgQE/100 mL), while these compounds were detected only in trace amounts in the microgreen–apple juices (<LOQ or <LOD). These results are consistent with the study by Lopez-Rodulfo, Tsochatzis, Stentoft, Martinez-Carrasco, Bechtner and Martinez [31], which reported limited and poor migration of flavonols from apples into juice during the squeezing process. Both kaempferol sinapoyl polyglycosides were detected at low concentrations in broccoli–apple juice. Catechin (37.99 µg/100 mL), epicatechin (32.11 µg/100 mL), and total procyanidins (170.83 µgPB2/100 mL) were found at high concentrations in apple juice, as well as in microgreen–based juices, primarily due to the contribution of apple juice in the blends. These compounds are the main flavonoids in apples and have previously been reported at high levels in both apple fruits [28,66] and juice [31,67]. However, their migration from apples to juice strongly depends on the degree of polymerization [31], which explains the absence of polymeric forms (trimers, tetramers, etc.). Dihydrochalcones are also characteristic apple compounds, and their presence in microgreen–apple juices directly depends on their concentration in the apple juice. Phloretin and 3-hydroxyphloretin were detected at low concentrations in the control apple juice (<1 µgPH/100 mL), while the contents of their glycosides were significantly higher in both the apple juice (2.94–4.70 µgPH/100 mL) and microgreen–apple juices (0.96–2.57 µgPH/100 mL). These results confirmed better solubility and migration of dihydrochalcone glycosides into apple juice during processing than of their aglycones.

Apigenin *C*-glycosides were detected only in the red beet–apple juice as typical compounds derived from red beet microgreens [3,58] and represented the predominant group of phenolic compounds in this juice (359.73 µgIsV/100 mL). Notable migration of these compounds from red beet microgreens into juice has been reported in our previous study [3]. Quantification confirmed that the total amount of cytisoside derivatives (230.17 µgIsV/100 mL) was significantly higher than vitexin derivatives (126.46 µgIsV/100 mL), primarily due to the contribution of 2″-hexosyl-6″-acetyl cytisoside (71.42 µgIsV/100 mL), 2″-hexosyl cytisoside (49.78 µgIsV/100 mL), and 2″-pentosyl cytisoside (57.83 µgIsV/100 mL). The content of all the (*iso*)vitexin derivatives in the red beet–apple juice ranged from 24.68 µgIsV/100 mL (2″-hexosyl vitexin) to 35.24 µgIsV/100 mL (2″-pentosyl-6″-malonyl vitexin).

3.4. Total Phenolic Content and Antioxidant Properties of Cold-Pressed Microgreen–Apple Juices

The total phenolic content and potential antioxidant activity of cold-pressed microgreen–apple juices were evaluated using screening assays and are presented in Figure 4.

In multicomponent samples such as the microgreen–apple juices, the Folin–Ciocalteu (F–C) reagent can be reduced by various non-phenolic compounds, resulting in an apparent overestimation of the total phenolic content. Therefore, this method reflects the overall reducing capacity of the samples rather than exclusively measuring phenolic compounds [69]. Considering this observation, obtained TPC values in the microgreen-based juices ranged from 33.70 ± 0.32 mg GAE/100 mL (RB-AJ) to 54.89 ± 0.54 mg GAE/100 mL (BC-AJ) (Figure 4a). The obtained results are consistent with the total phenolic content determined by UHPLC-QToF-MS analysis. These TPC values are comparable to those reported by other

authors, such as 51 mg GAE/100 mL for untreated *Alternanthera sessilis* microgreen juice mixed with honey and ginger [17].

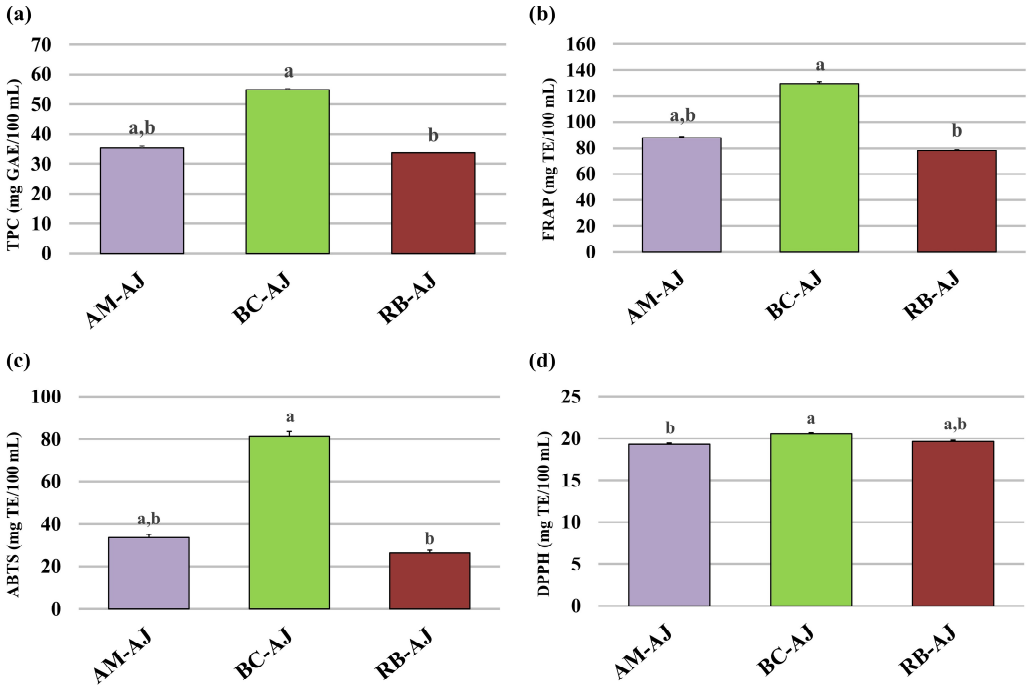


Figure 4. Screening assay for the evaluation of the antioxidant potential of the microgreen–apple juices: the total phenolic content (**a**); ferric reducing antioxidant power (FRAP) (**b**); ABTS•+ scavenging radical activity (**c**); and DPPH• radical scavenging activity (**d**). The different lowercase letters denote significant differences among the microgreens–apple juices, according to Dunn's test ($p < 0.05$). Abbreviations: "RB-AJ", red beet microgreens–apple juice; "BC-AJ", broccoli microgreens– apple juice; and "AM-AJ", amaranth microgreens–apple juice.

Free radicals are known to be detrimental to human health and are associated with the development of various diseases, including cardiovascular and neurodegenerative disorders. In contrast, antioxidants, such as polyphenolic compounds, betalains, and glucosinolates, neutralize free radicals and protect cells from their harmful effects. The aim of this study was to evaluate the potential antioxidant activity of microgreen–apple juices using screening FRAP, DPPH, and ABTS assays, which are based on different mechanisms of action of bioactive compounds against free radicals. Figure 4b–d present the antioxidant activity of microgreen-based juices. Significant differences ($p < 0.05$) were observed among the samples, with the highest antioxidant potential for broccoli–apple microgreens for all of the assays. The tested juices demonstrated a strong capacity to reduce the $[Fe^{3+}\text{-}(TPTZ)_2]^{3+}$ complex, indicating high reduction potential. The highest FRAP value was recorded for the BC-AJ (129.25 ± 1.63 mg TE/100 mL), followed by the AM-AJ (87.81 ± 0.97 mg TE/100 mL), and the RB-AJ (77.74 ± 1.12 mg TE/100 mL) (Figure 4b). These results were in agreement with TPC results, as well as with results previously reported for cold-pressed microgreen juices [3]. All of the microgreen–apple juices also exhibited good scavenging activity for ABTS radical, ranging from 81.45 ± 2.21 mg TE/100 mL (BC-AJ) to 26.47 ± 1.20 mg TE/100 mL (RB-AJ) (Figure 4c). In contrast, all samples showed poor scavenging activity for DPPH radicals, likely due to weaker interactions with this less polar radical (Figure 4d).

3.5. Sensory Analyses of Microgreen–Apple Juices

The visual appearance of the microgreen–apple juices and results of their sensory analyses are shown in Figure 5.

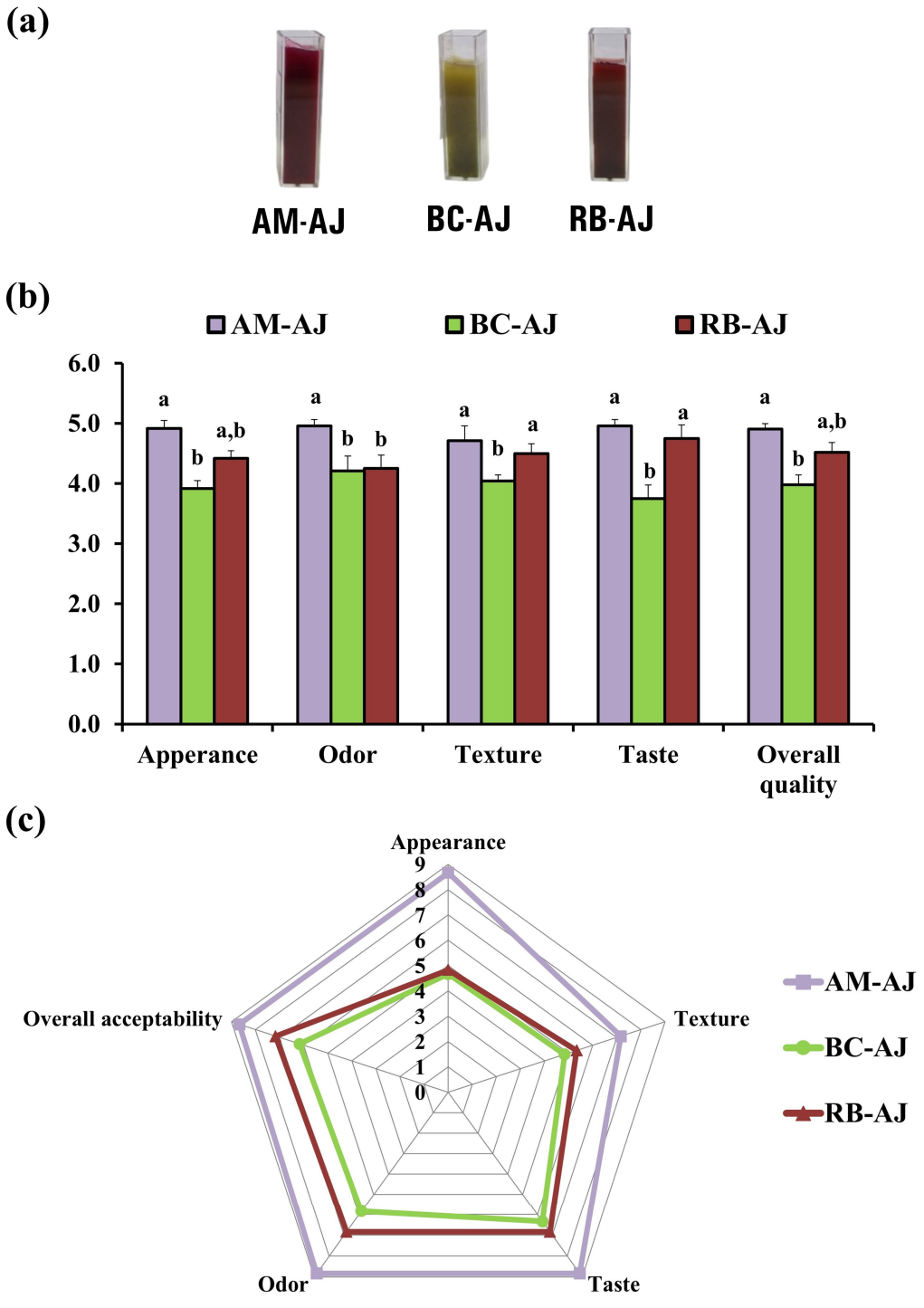


Figure 5. The visual appearance of the microgreen–apple juices (**a**), the sensory quality score (**b**), and the sensory radar chart for the likeability testing of the microgreen–apple juices (**c**). The different lowercase letters denote significant differences among the microgreen–apple juices, according to Dunn's test ($p < 0.05$). Abbreviations: "RB-AJ", red beet microgreens–apple juice; "BC-AJ", broccoli microgreens–apple juice; and "AM-AJ", amaranth microgreens–apple juice.

The results of the sensory quality evaluation are presented in Figure 5b. The microgreen-based juices exhibited significant differences ($p < 0.05$) for appearance and over-

all quality (AM-AJ and BC-AJ), for odor (AM-AJ and BC-AJ; AM-AJ and RB-AJ), and for texture and taste (AM-AJ and BC-AJ; RB-AJ and BC-AJ). The highest score for appearance was recorded for the AM-AJ (4.92), which was described as having an attractive pink–purple color. This can be attributed to its high betalain content, as previously reported [3]. In addition, the RB-AJ exhibited a similar but darker red–purple hue, reminiscent of mature beetroot, while the BC-AJ received a lower score (3.92) due to non-uniform coloration ranging from dark to bright green (Figure 5a). All of the samples were characterized by acceptable aroma profiles, with reduced earthy notes in amaranth–apple juice and red beet–apple juice, and a less pronounced cruciferous aroma in broccoli–apple juice. The addition of apple juice obviously contributed to a reduction in the intensity of inherent microgreen aromas. Although the BC-AJ received the lowest aroma score (4.21), likely due to mild cabbage-like notes and slight turbidity, this did not substantially affect its overall acceptability. Texture scores were lowest for the BC-AJ (4.21), followed by the RB-AJ (4.50) and AM-AJ (4.71), with evaluators noting slight turbidity and minor sedimentation in all samples. These characteristics are typical of cold-pressed cloudy juices and may be associated with higher polyphenol content [70]. The taste scores were generally high, ranging from 3.75 (BC-AJ) to 4.96 (AM-AJ), with no pronounced negative attributes. The BC-AJ exhibited a slightly sour and cabbage-like taste, likely due to sulfur compounds [71], whereas the AM-AJ and RB-AJ lacked undesirable earthy notes. The overall taste profile was influenced by apple-derived compounds, such as procyanidins and dihydrochalcones, as well as the presence of sugars and organic acids (malic acids), contributing to balanced sweetness and acidity [72]. The overall quality ratings ranged from "very good" for the BC-AJ (3.98) to "excellent" for the AM-AJ (4.90) and the RB-AJ (4.52), representing an improvement compared to our previous study [3]. These findings indicate that the addition of apple juice positively influenced the sensory quality and acceptability of the microgreen–apple juices.

The consumer preference for microgreen-based juices is presented in Figure 5c. The results were consistent with the quality rating method (Figure 5b), with the AM-AJ receiving the highest overall scores. In general, the appearance and texture were the main attributes contributing to lower ratings of microgreen-based juices. The AM-AJ achieved a significantly higher appearance score (8.67) compared to the other samples. The overall acceptability ranged from "like extremely" for the AM-AJ (8.67) to "like slightly" for the BC-AJ (6.17). Compared with our previous study on cold-pressed microgreen juices [3], the addition of apple juice improved consumer acceptance, as reflected in higher overall acceptability scores. The consumers particularly appreciated the AM-AJ for its balanced sweetness and acidity, as well as a mild metallic sensation during and after consumption. In contrast, the BC-AJ received lower scores due to the presence of visible particles, slight cabbage-like notes, and an aftertaste described as sweetish, bitter, and astringent. These sensory characteristics can be linked to the presence of polyphenols and glucosinolates, which contribute to bitterness, astringency, and pungency [3,20], while apple-derived procyanidins further influence astringency and bitterness [72]. Similar trends have been reported for beverages based on *Brassica juncea* L. microgreen juice [41]. Overall, apple juice, with its favorable sugar-to-acid ratio, contributed to masking undesirable grassy, earthy, and sulfurous notes in microgreen-based juices, while dihydrochalcones enhanced sweetness perception. The developed microgreen-based juices demonstrated good consumer acceptability and can be considered as potential functional beverages with favorable sensory properties.

4. Limitations

Cold-pressed juices are characterized by higher levels of nutrients and bioactive compounds, as well as improved sensory properties; however, this processing method also

presents certain limitations, including limited applicability, high production costs, and a shorter shelf life. The present study has an experimental character and was conducted under laboratory conditions, which may limit its industrial production due to constraints related to raw material availability (microgreens) and storage conditions.

The microgreen–apple juices were mixed in a specific ratio (51:49, v/v) without prior systematic optimization. Taking this into account, the prepared blends represent a single point in a broader optimization space for each of the prepared blend juices (RB–AJ, BC–AJ, and AM–AJ), which is a limitation of this study. To obtain an optimal balance between bioactivity and acceptability for each of the reported microgreen–apple juice blends, systematic optimization and a design of experiments approach should be included in future studies.

In addition, only one apple variety (Golden Delicious), the most commonly consumed in Serbia, was used; therefore, future studies should explore the use of other apple varieties in juice formulations. The antioxidant potential of the developed microgreen–apple juices was evaluated using three screening assays, while further advanced in vitro and in vivo studies are required to confirm their functional properties.

Finally, sensory evaluation was conducted with a panel of 55 students. Although this group provides useful preliminary insights into product acceptability, it does not fully represent the broader consumer population in terms of age, cultural background, and experience with similar products. Consequently, in further studies, sensory evaluation involving a more diverse group of consumers is recommended.

5. Conclusions

In summary, the developed microgreen–apple juices demonstrated high overall quality and good consumer acceptability, particularly the amaranth–apple juice. All samples exhibited acceptable aroma profiles, high taste scores without pronounced negative attributes, and typical sedimentation associated with cold-pressed cloudy juices. Sensory evaluation indicated that the blending of microgreens and apple juices effectively suppressed earthy notes in amaranth and red beet juices and reduced the intensity of the cruciferous aroma in broccoli-based juice. The addition of apple juice influenced the physicochemical properties of the blends, decreasing pH and increasing total soluble solids (TSS), contributing to the development of decreased-calorie beverages. Comprehensive UHPLC Q-ToF MS analysis identified 71 bioactive compounds, including phenolics, betalains, and one glucosinolate. Phenolic compounds were predominant and were quantified or semi-quantified to determine their distribution in the microgreen–apple juices. Based on the profiling, multivariate statistical analysis, and semi-quantification, the results indicate that the dominant compounds varied among samples and were mostly derived from the respective microgreens. Hydroxybenzoic acid, sinapic acid, and their derivatives were most abundant in the broccoli–apple juice; phenolic acid diglycosides and hydroxycinnamoyl–isocitric acid derivatives predominated in the amaranth–apple juice; and apigenin C-glycosides (vitexin and cytisoside derivatives) were characteristic of the red beet–apple juice. In addition to phenolics, the amaranth– and red beet–apple juices contained betalains, whereas the broccoli–apple juice contained trace levels of a glucoraphanine, which was most probably degraded. All of the microgreen–apple juices also contained apple-derived flavan-3-ols, procyanidins, dihydrochalcones, and certain flavonols, transferred during processing. All of the microgreen–apple juices exhibited promising antioxidant potential, with differences attributable to variations in profiles of bioactive compounds. Overall, the developed cold-pressed microgreen–apple juices can be classified as novel, low-calorie, and highly sensory-acceptable beverages, rich in diverse bioactive compounds derived from both apple and microgreens. In future studies, it will be necessary to define the optimal ratio of microgreens to apple juice using a design-of-experiments approach in order to achieve a balance

between bioactivity and sensory acceptability for each of the investigated microgreen–apple juice blends, as well as to conduct advanced in vitro and in vivo studies to additionally evaluate their functionalities.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/beverages12060073/s1. Table S1: Regression equation and other accompanying parameters of standards used for quantification or semi-quantification of (tentatively) identified phenolic compounds and glucoraphanin in analyzed microgreen-based juices.

Author Contributions: Conceptualization: S.D.B., D.D.M., S.M.L. and V.A.N.; methodology: S.D.B., D.D.M. and U.M.G.; software: S.D.B. and D.D.M.; validation: D.D.M. and U.M.G.; formal analysis: S.D.B., D.D.M., U.M.G., J.M.M., V.B.Đ. and S.M.L.; investigation: S.D.B. and D.D.M.; resources: M.B.P., V.B.Đ. and V.A.N.; data curation: S.D.B. and D.D.M.; writing—original draft preparation: S.D.B. and D.D.M.; writing—review and editing: S.D.B., D.D.M., S.M.L., M.B.P., J.M.M., U.M.G. and V.A.N.; visualization: S.D.B., D.D.M. and U.M.G.; supervision: S.M.L., M.B.P., U.M.G. and V.A.N.; project administration: S.D.B., S.M.L. and V.A.N.; funding acquisition: M.B.P., V.B.Đ. and V.A.N. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: All sensory tests with participants were conducted in accordance with the Code of Professional Ethics of the University of Belgrade. This study follows the Serbian Law on the Protection of Personal Data (Official Gazzet of the Republic of Serbia, 87/2018). Based on the exemption statement issued by the Ethics Committee of the Faculty of Agriculture, University of Belgrade, Serbia (No. 22/268), this study did not require formal ethical approval, since no ethically sensitive procedures were involved.

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding authors.

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Conflicts of Interest: The authors declare no competing financial interests.

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