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Selected Antioxidants in Organic vs. Conventionally Grown Apple Fruits

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Abstract: The apple (*Malus domestica* Borkh.) is one of the most widely cultivated temperate fruits globally, gaining scientific interest as a rich source of antioxidants with a demonstrated beneficial human health impact. Since a growing number of consumers are increasingly seeking safe and healthy food options, alternative fruit production systems such as organic farming, and their potential to provide safe and nutritious foods, have been gaining increasing attention in the last decades. The aim of the presented study was, therefore, to analyse and to compare the concentrations of selected health-promoting antioxidants, such as phenolic acids, flavonols, and vitamin C, in fruits of three apple cultivars (Champion, Gala, and Idared) grown in conventional and certified organic orchards in Poland. All analyses were performed using the high-performance liquid chromatography (HPLC) method. Organic apples tested within the study, compared to the conventionally grown ones, were characterised by significantly higher concentrations of phenolic acids (av. >31%) and flavonols (av. >66%) with the identified differences being consistent in all three cultivars and two seasons. The greatest production system effect was observed in the case of Idared. Significant cultivar and production season effects on the concentration of the measured fruit phenolics, with strong interactions between the two factors, were also identified. Vitamin C content in the fruits was strongly dependent on the year-to-year differences in the fruit growing conditions. The study suggests that the organic production system has a potential to provide apple fruits rich in selected health-promoting phenolic antioxidants.

Keywords: apple; *Malus domestica*; organic production; bioactive compounds; HPLC; phenolic compounds; phenolic acids; flavonols; vitamin C

1. Introduction

Apple (*Malus domestica* Borkh.) is currently the most widely cultivated temperate fruit, with a global production of over 76 million tonnes per year [1]. Besides fresh consumption, apple fruits are also processed into juices, concentrates, purees, ciders, and many other products [2]. They reached such a position due to their high yielding, overall abundance, accessibility on the market, long storage capacity, and their taste and rich phytochemical profile. Due to being widely consumed and available in all seasons, apples became one the most important fruit sources of health-promoting phenolic compounds in the Western diet [3]. This resulted in the growing scientific interest in these fruits' potential to enhance human health.

A number of research studies have linked apple consumption to the reduced risk of various non-communicable diseases, including certain cancers, type II diabetes, and cardiovascular disease [3–5].

Moreover, apple fruit consumption was shown to be associated with positive effects on cognitive functions, weight management, bone density, pulmonary function, and gastrointestinal health [5,6]. The in vitro experiments reported the antioxidant, cell signaling, and antiproliferative mechanisms of apple extracts action [4]. Many of these effects were associated with phenolics and dietary fibre in apples [7]. The available evidence links consumption of fruit phenolics to the prevention of cancers, neurodegenerative diseases, type 2 diabetes, osteoporosis, cardiovascular diseases, and many other non-communicable diseases through and beyond the oxidative stress modulation [8]. It is, therefore, considered that phenolics significantly contribute to the overall health-promoting effect of vegetable-rich and fruit-rich diets [9].

Increasing public concerns about the negative health impact of synthetic pesticides widely used in conventional agriculture and horticulture became one of significant drivers of the demand for fruit and vegetables coming from alternative systems. One such system gaining popularity in recent decades is organic farming [10–13]. Organic producers are not allowed to use synthetic pesticides in order to limit both the environmental impacts of production inputs and to decrease the risk of consumers' exposure to pesticide residues in fruit and vegetables [14]. Instead, organic farms rely mainly on natural plant protection and preventive measures (i.e., more resistant or tolerant cultivars, mechanical weed control, and biological pest and disease control measures). Only a limited number of microbial or plant extract-based and mineral (i.e., S-based and Cu-based) plant protection products are available for organic farmers [13]. Moreover, organic and non-organic production differ in respect to fertilisation protocols (organic vs. mineral synthetic fertilizers) as well as crop rotation systems [15,16]. Such contrasting practices applied in organic and non-organic systems may have an impact on yield, but also on plants' metabolism, which leads to differences in the crops' composition. Recently published research has indicated that replacing synthetic with natural fertilizers in agricultural production affects the profile of secondary metabolites (including phenolics) in plant tissues by changing the protein expression [17,18]. As reported in the recently published meta-analysis [19], not only the profile, but also the concentrations of plant phenolics may differ significantly between organically and non-organically cultivated fruits and vegetables, with organic ones being, on average, characterized by higher concentrations of these antioxidants. However, it should be pointed out that many of the organic vs. conventional comparison studies carried out, so far, targeted only one cultivar of selected species and/or were undertaken in one vegetation season, and, therefore, did not allow the authors to consider the effects of genetic and season (weather/environment)-related factors on the composition of organic and non-organic fruits and vegetables. Among the published research on the antioxidants' profiles of organic vs. non-organic crops, only a few looked into apples, and an even more limited number focused on the profiles of phenolic acids and flavonols, which constitute 3%-41% of all apple polyphenols [20]. Considering the above, carrying out research on these bioactive compounds' profiles in apple fruits grown in an organic horticultural system, may be of relevance and of interest for the producers and the consumers. The aim of the study presented in this paper was, therefore, to analyze and to compare the concentrations of phenolic acids, flavonols, and vitamin C in fruits of three apple cultivars harvested in two consecutive growing seasons from selected organic and conventional orchards located in Poland.

2. Materials and Methods

2.1. Plant Material

The study was carried out at the Warsaw University of Life Sciences (Poland). Fruits of three apple cultivars (Champion, Gala, and Idared) were harvested in the 2017 and 2018 vegetation season from certified organic and conventional orchards located in central Poland (Mazovia region). Orchards selected for the study were characterized by a comparable agricultural environment (i.e., soil and climate conditions). They were also matched according to rootstock, trees age, and trailing systems used. Apple fruit samples of ≥3 kg from each orchard were harvested at the same stage of ripening, in three replicates, and immediately transported to the laboratory of the Division of Organic Foods

(WULS-SGGW). The fruits were sieved and cut into pieces. Fresh sub-samples of each sample were used for dry matter determination. The remaining material was freeze-dried using Labconco freeze-drier (−45 °C, 0.11 mBar), ground in a laboratory mill A-11, and stored at −80 °C before further analyses.

2.2. Chemicals

Acetonitrile of high-performance liquid chromatography (HPLC) purity (Sigma-Aldrich, Poland), deionized water (Sigma-Aldrich, Poland), sodium acetate of HPLC purity, (Merck, Poland), methanol of HPLC purity (Merck, Poland), ortho-phosphoric acid 99.9%, metaphosphoric acid 99.9% (Chempur, Poland), phenolics standards (purity 99.0–99.9%): gallic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, quercetin-3-*O*-rutinoside, kempferol-3-*O*-glycoside, quercetin (Sigma-Aldrich, Poland), L-ascorbic acid (L-Asc), and dehydroascorbic acid (DHA) standards with 99% purity (Sigma-Aldrich, Poland).

2.3. Dry Matter Content

Dry matter was determined before freeze drying, as described by Isaac and Maalekuu (2013) [21]. Empty glass beakers were weighed, filled with fresh apple (flesh and skin) material, and weighed again. Samples were dried at 105 °C, 1013 hPa, for 48 h in an FP-25W Farma Play (Poland). After 48 h, samples were cooled to 21 °C and weighed. Dry matter content was calculated (in g/100 g f.w.) based on a mass difference.

2.4. Phenolic Acids and Flavonols Extraction and Identification

Polyphenols (phenolic acids and flavonols) were analysed using High Performance Liquid Chromatography (HPLC) method, as previously described by Kazimierczak et al. (2019) [22]. The analyses were done with Shimadzu equipment (USA Manufacturing Inc, USA, two LC-20AD pumps, CBM-20A controller, a CTD-20AC oven, SIL-20AC autosampler, UV/Vis SPD-20AV detector). A 100-mg freeze-dried apple fruit sample was mixed with 5 mL of 80% methanol, shaken on a Micro-Shaker 326 M (Poland), and incubated in an ultrasonic bath at 30 °C for 10 min. Then the sample was centrifuged (3450 g, 12 min, 2 °C), supernatant was collected, and 500 µl of the supernatant was injected onto the HPLC Synergi Fusion-RP 80i Phenomenex column (250 mm × 4.60 mm). Polyphenols were separated under gradient conditions with a flow rate of 1 mL min−1 by applying an aqueous solution of 10% (*v*/*v*) acetonitrile (phase A) and 55% (*v*/*v*) acetonitrile (phase B), both acidified by ortho-phosphoric acid to pH 3.0. Time of the analysis was 38 min. The phases changed as follows: 1.00–22.99 min 95% phase A and 5% phase B, 23.00–27.99 min 50% phase A and 50% phase B, 28.00–35.99 min 80% phase A and 20% phase B, and 36.00–38.00 min 95% phase A and 5% phase B. The wavelengths used for detection were as follows: 250 nm for flavonols and 370 nm for phenolic acids. Identification of individual phenolics was based on Sigma-Aldrich and Fluka external standards with a purity of 99.00–99.99%. HPLC chromatograms presenting time of retention and profiles of the identified phenolic acids and flavonols are presented in Figures 1 and 2, respectively. Concentration of compounds was calculated based on standard curves and applied dilution coefficients.

2.5. Vitamin C

Vitamin C in apples was determined by the high performance liquid chromatography (HPLC) method, as previously described by Kazimierczak et al. (2019) [22], using Shimadzu equipment characterized in the previous chapter. A 100-mg freeze-dried apple fruit sample was extracted with 5% metaphosphoric acid. The samples were mixed by a vortex mixer, incubated in an ultrasonic bath (15 min, 20 °C), and centrifuged (6000 rpm, 10 min, 0 °C). The supernatant (100 µl) was injected onto the Phenomenex Hydro 80-A RP column (250 × 4.6 mm). The analysis parameters were as follows: mobile phase of 50 mM phosphate buffer (pH 4.4) and 0.1 mM sodium acetate, analysis time of 18 min, and detection wavelength of 255–260 nm. L-ascorbic acid (L-Asc) and dehydroascorbic acid (DHA) were identified based on Fluka and Sigma-Aldrich (Warsaw, Poland) standards with 99% purity.

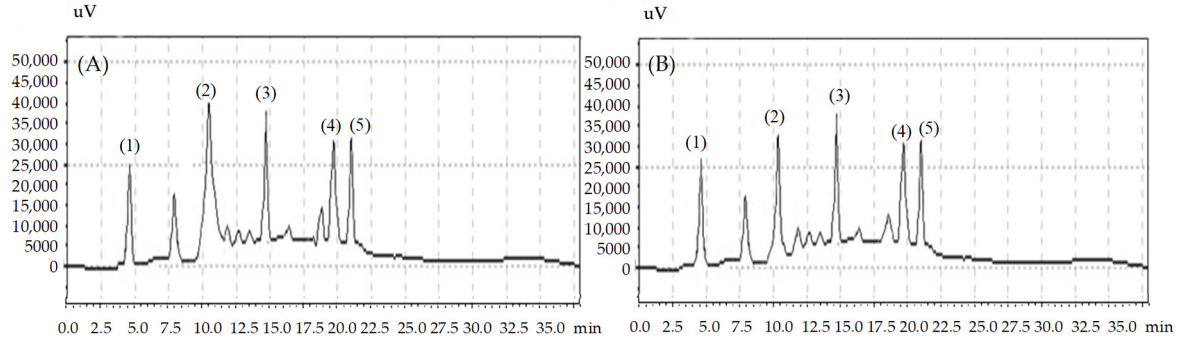

Figure 1. Chromatograms showing retention times and peak areas for phenolic acids in organic (**A**) and conventional (**B**) Gala apples, (1) gallic acid, (2) chlorogenic acid, (3) caffeic acid, (4) *p*-coumaric acid, and (5) ferulic acid.

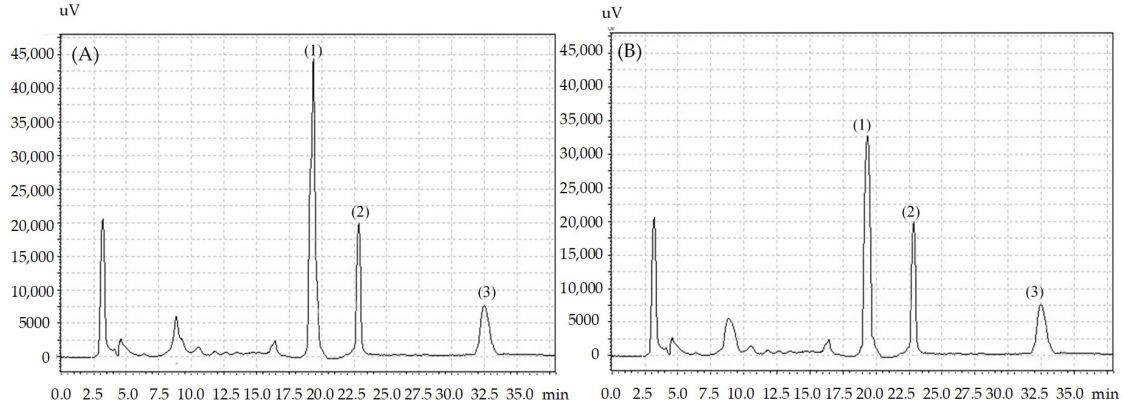

Figure 2. Chromatograms showing retention times and peak areas for flavonols in organic (**A**) and conventional (**B**) Gala apples, (1) quercetin-3-*O*-rutinoside, (2) kaempferol-3-*O*-glucoside, and (3) quercetin.

2.6. Statistical Analyses

The main effects of the (a) horticultural production system, (b) vegetation season, and (c) apple cultivar on the analysed apple fruit compositional parameters as well as interactions between the three factors were assessed using three-factor ANOVA (see Tables 1–3). In addition, for further explanation of the identified interactions, two-factor ANOVA, with the horticultural system and the cultivar as factors, followed by post hoc Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$), was performed separately for each of the two vegetation seasons (Table 4, Figures 3–5). All the analyses were carried out in R programme, with an 'nlme' statistical package [23]. Data normality was assessed in R using D'Agostino-Pearson and the qqnorm tests. In addition, Pearson product-moment correlation analyses between concentrations of individual phytonutrients were performed in R, and the correlation matrix figure (Figure 6) was produced using 'corrplot' package.

Table 1. The main effect of (and interaction between) the production system (conventional and organic), cultivar (Champion, Gala, Idared) and harvest year (2017, 2018) on the dry matter content (g/100 g f.w.) and vitamin C (mg/100 g f.w.) concentrations (mean ± SD) in apple fruit.

	Dry Matter	Vitamin C	Dehydroascorbic Acid	L-Ascorbic Acid
System (SYS)				
Conventional	12.88 ± 1.07	12.73 ± 6.59	1.24 ± 0.40	11.49 ± 6.47
Organic	14.48 ± 1.20	12.98 ± 3.53	1.96 ± 0.80	11.02 ± 4.18
Cultivar (CV)				
Champion	14.24 ± 1.47	12.98 ± 3.24	1.68 ± 0.35	11.30 ± 3.13
Gala	13.50 ± 1.57	12.28 ± 7.59	1.81 ± 1.12	10.47 ± 7.93

Table 1. *Cont.*

	Dry Matter	Vitamin C	Dehydroascorbic Acid	L-Ascorbic Acid
Idared	13.30 ± 0.98	13.30 ± 4.21	1.30 ± 0.36	11.99 ± 4.23
Year (YR)				
2017	14.14 ± 1.69	8.47 ± 3.07	1.77 ± 0.93	6.70 ± 3.02
2018	13.22 ± 0.80	17.23 ± 2.44	1.42 ± 0.39	15.81 ± 2.51
ANOVA *p*-values				
SYS	0.000	NS	0.001	0.027
CV	0.000	0.000	0.000	0.000
YR	0.021	0.000	0.050	0.000
SYS × CV	0.000	0.000	0.000	0.000
SYS × YR	0.004	0.000	0.002	0.000
CV × YR	0.000	0.000	0.006	0.000
SYS × CV × YR	NS	0.000	0.001	0.000

Table 2. The main effect of (and interaction between) the production system (conventional and organic), cultivar (Champion, Gala, Idared) and harvest year (2017, 2018) on the concentrations (mean ± SD) of phenolic acids (mg/100g f.w.) in apple fruit.

	Phenolic Acids (sum)	Gallic Acid	Chlorogenic Acid	Caffeic Acid	*p*-Coumaric Acid
System (SYS)					
Conventional	13.3 ± 2.3	0.94 ± 0.92	10.8 ± 1.8	0.58 ± 0.18	0.97 ± 0.52
Organic	17.5 ± 1.6	0.84 ± 0.53	14.8 ± 2.0	0.71 ± 0.22	1.14 ± 0.62
Cultivar (CV)					
Champion	16.2 ± 2.0	1.14 ± 1.08	13.4 ± 2.3	0.72 ± 0.23	0.92 ± 0.72
Gala	14.9 ± 1.7	0.63 ± 0.21	12.1 ± 1.6	0.62 ± 0.26	1.46 ± 0.32
Idared	15.2 ± 4.4	0.91 ± 0.64	12.9 ± 4.0	0.59 ± 0.08	0.80 ± 0.37
Year (YR)					
2017	15.4 ± 2.7	0.50 ± 0.08	12.8 ± 2.6	0.79 ± 0.15	1.33 ± 0.31
2018	15.4 ± 3.2	1.28 ± 0.90	12.9 ± 3.0	0.50 ± 0.15	0.79 ± 0.64
ANOVA *p*-values					
SYS	0.000	0.003	0.000	0.004	0.002
CV	0.001	0.000	0.001	0.000	0.000
YR	NS	0.001	NS	0.005	0.002
SYS × CV	0.000	0.000	0.000	0.000	0.000
SYS × YR	NS	0.001	NS	NS	NS
CV × YR	0.000	0.000	0.000	0.000	0.000
SYS × CV × YR	0.038	0.000	NS	0.017	0.000

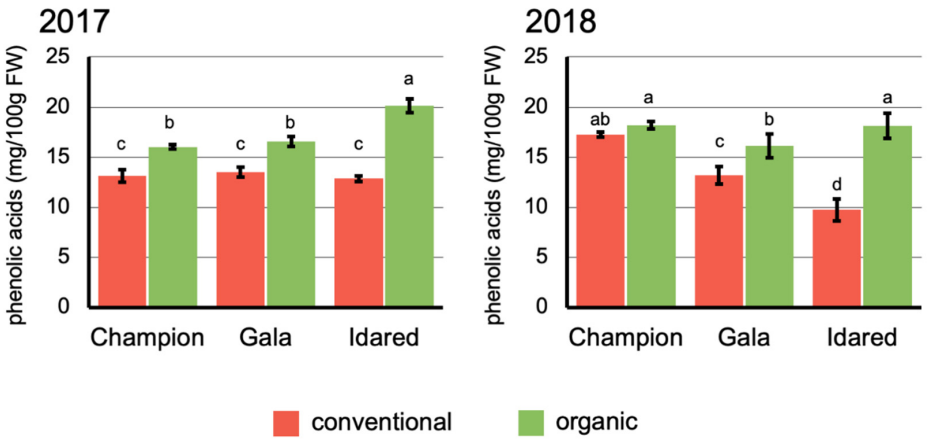

Figure 3. Concentrations (mean and SD) of phenolic acids (sum) in Champion, Gala, and Idared apple fruits collected from organic and conventional orchards in 2017 and 2018 growing seasons. Within each season and group of compounds, bars marked with the same letters are not significantly different at $p < 0.05$.

Table 3. The main effect of (and interaction between) the production system (conventional and organic), cultivar (Champion, Gala, Idared) and harvest year (2017, 2018) on the concentrations (mean ± SD) of flavonols (mg/100g f.w.) in apple fruit.

	Flavonols (sum)	Quercetin-3-*O*-Rutinozide	Kempferol-3-*O*-Glycoside	Quercetin
System (SYS)				
Conventional	3.35 ± 1.83	3.00 ± 1.85	0.16 ± 0.03	0.19 ± 0.02
Organic	5.57 ± 1.41	5.16 ± 1.45	0.19 ± 0.11	0.21 ± 0.03
Cultivar (CV)				
Champion	4.31 ± 2.02	3.94 ± 1.99	0.16 ± 0.04	0.21 ± 0.03
Gala	4.83 ± 0.89	4.44 ± 0.86	0.20 ± 0.10	0.20 ± 0.04
Idared	4.23 ± 2.68	3.87 ± 2.73	0.17 ± 0.09	0.19 ± 0.02
Year (YR)				
2017	3.53 ± 2.18	3.09 ± 2.13	0.22 ± 0.08	0.22 ± 0.03
2018	5.39 ± 1.16	5.07 ± 1.18	0.13 ± 0.04	0.18 ± 0.01
ANOVA *p*-values				
SYS	0.000	0.000	0.003	0.000
CV	0.000	0.000	0.000	0.000
YR	0.004	0.004	0.004	0.001
SYS × CV	0.000	0.000	0.000	0.000
SYS × YR	0.000	0.001	0.000	0.000
CV × YR	0.000	0.000	0.000	0.000
SYS × CV × YR	0.000	0.000	0.000	0.000

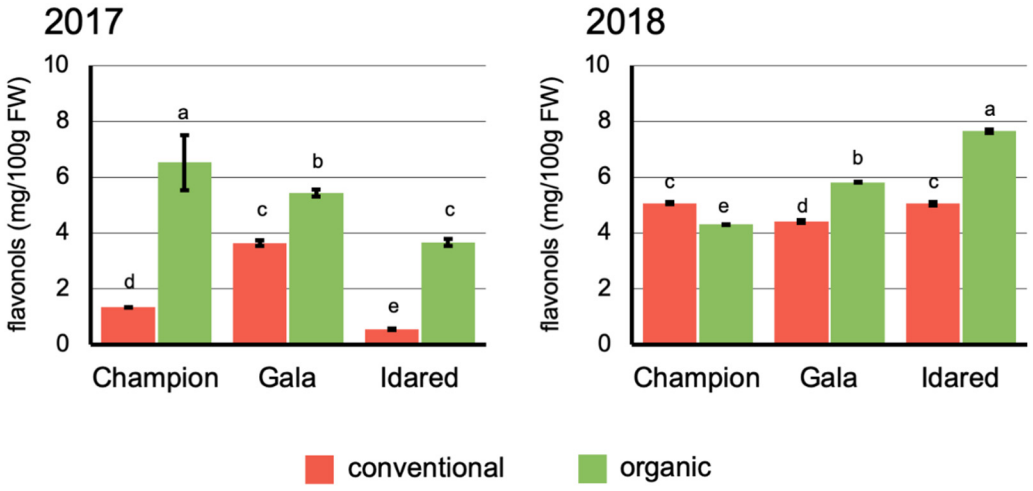

Figure 4. Concentrations (mean and SD) of flavonols (sum) in Champion, Gala, and Idared apple fruits collected from organic and conventional orchards in 2017 and 2018 growing seasons. Within each season and group of compounds, bars marked with the same letters are not significantly different at $p < 0.05$.

Table 4. Effect of (and interaction between) production system (conventional and organic) and cultivar (Champion, Gala, Idared) on the dry matter content (g/100 g f.w.), vitamin C (mg/100g f.w.), phenolic acids (mg/100g f.w.), and flavonols (mg/100 g f.w.) concentrations (mean ± SD) in apple fruit (2017–2018). Within rows, mean values followed by the same letter are not significantly different at $p < 0.05$.

Parameter	Year	Champion		Gala		Idared		ANOVA *p*-Values		
		CONV	ORG	CONV	ORG	CONV	ORG	SYS	CV	SYS × CV
Dry matter	2017	14.7 ± 0.9b	16.2 ± 0.1a	11.5 ± 0.3d	15.1 ± 0.6b	12.6 ± 0.2c	14.7 ± 0.3b	0.010	0.000	0.018
	2018	13.3 ± 0.1b	12.7 ± 0.1c	12.7 ± 0.3c	14.7 ± 0.2a	12.4 ± 0.1c	13.4 ± 0.3b	0.012	0.000	0.000
Vitamin C (sum)	2017	9.8 ± 0.2b	10.5 ± 0.1b	3.6 ± 0.3d	7.1 ± 0.4c	6.9 ± 1.0c	12.8 ± 0.6a	0.005	0.000	0.000
	2018	17.6 ± 0.2b	14.0 ± 0.2e	21.9 ± 0.2a	16.5 ± 0.3cd	16.5 ± 0.4d	17.0 ± 0.2c	0.003	0.000	0.000
Dehydroascorbic acid	2017	1.37 ± 0.0bc	1.98 ± 0.13b	0.89 ± 0.05c	3.50 ± 0.83a	1.12 ± 0.03c	1.80 ± 0.10b	0.016	0.017	0.002
	2018	2.03 ± 0.03a	1.33 ± 0.00c	1.13 ± 0.03d	1.73 ± 0.08b	0.89 ± 0.04e	1.40 ± 0.06c	0.025	0.000	0.000
L-ascorbic acid	2017	8.47 ± 0.19b	8.52 ± 0.12b	2.76 ± 0.20d	3.62 ± 0.44d	5.82 ± 0.98c	11.01 ± 0.53a	0.014	0.000	0.000
	2018	15.60 ± 0.30b	12.60 ± 0.20d	20.70 ± 0.20a	14.80 ± 0.20c	15.60 ± 0.40b	15.60 ± 0.20b	0.003	0.000	0.000
Phenolic acids (sum)	2017	13.2 ± 0.6c	16.0 ± 0.2b	13.5 ± 0.5c	16.6 ± 0.5b	12.9 ± 0.3c	20.1 ± 0.7a	0.003	0.000	0.000
	2018	17.3 ± 0.2ab	18.2 ± 0.4a	13.2 ± 0.9c	16.1 ± 1.2b	9.7 ± 1.1d	18.1 ± 1.2a	0.019	0.000	0.000
Gallic acid	2017	0.49 ± 0.01bc	0.62 ± 0.04a	0.52 ± 0.01b	0.55 ± 0.07b	0.40 ± 0.01d	0.44 ± 0.02cd	NS	0.000	0.011
	2018	2.92 ± 0.11a	0.51 ± 0.02d	0.47 ± 0.01d	0.98 ± 0.01c	0.86 ± 0.09c	1.92 ± 0.12b	0.011	0.000	0.000
Chlorogenic acid	2017	10.7 ± 0.6d	12.5 ± 0.2c	10.6 ± 0.4d	14.1 ± 0.3b	10.9 ± 0.2d	17.7 ± 0.6a	0.002	0.000	0.000
	2018	13.7 ± 0.1bc	16.6 ± 0.3a	11.0 ± 0.9d	12.9 ± 1.1c	7.9 ± 1.0e	15.2 ± 1.3ab	0.018	0.000	0.001
Caffeic acid	2017	0.69 ± 0.00c	1.02 ± 0.03a	0.86 ± 0.08b	0.87 ± 0.08b	0.59 ± 0.05d	0.70 ± 0.01c	0.028	0.000	0.002
	2018	0.41 ± 0.10cd	0.76 ± 0.09a	0.34 ± 0.02d	0.40 ± 0.06cd	0.60 ± 0.05b	0.49 ± 0.01bc	NS	0.000	0.000
p-Coumaric acid	2017	1.28 ± 0.01c	1.86 ± 0.05a	1.51 ± 0.14b	1.04 ± 0.15d	1.01 ± 0.02d	1.27 ± 0.11c	NS	0.000	0.000
	2018	0.20 ± 0.01f	0.33 ± 0.00e	1.42 ± 0.06b	1.86 ± 0.06a	0.41 ± 0.03d	0.51 ± 0.05c	0.008	0.000	0.000
Flavonols (sum)	2017	1.35 ± 0.00d	6.53 ± 0.98a	3.65 ± 0.10c	5.44 ± 0.12b	0.55 ± 0.03e	3.67 ± 0.13c	0.004	0.000	0.000
	2018	5.07 ± 0.03c	4.30 ± 0.01e	4.41 ± 0.05d	5.82 ± 0.01b	5.06 ± 0.06c	7.65 ± 0.07a	0.000	0.000	0.000
Quercetin-3-*O*-rutinozide	2017	0.97 ± 0.00d	6.08 ± 0.94a	3.35 ± 0.10c	4.84 ± 0.15b	0.18 ± 0.01e	3.14 ± 0.13c	0.004	0.000	0.000
	2018	4.69 ± 0.03d	4.03 ± 0.01e	4.04 ± 0.05e	5.52 ± 0.01b	4.78 ± 0.06c	7.38 ± 0.07a	0.000	0.000	0.000
Kempferol-3-*O*-glycoside	2017	0.16 ± 0.00bc	0.20 ± 0.04b	0.15 ± 0.00c	0.35 ± 0.03a	0.15 ± 0.03c	0.32 ± 0.01a	0.007	0.003	0.001
	2018	0.19 ± 0.00a	0.10 ± 0.00cd	0.19 ± 0.00a	0.10 ± 0.00c	0.11 ± 0.00b	0.10 ± 0.00d	0.000	0.000	0.000
Quercetin	2017	0.22 ± 0.00b	0.25 ± 0.00a	0.16 ± 0.00c	0.25 ± 0.00a	0.21 ± 0.00b	0.22 ± 0.00b	0.002	0.000	0.000
	2018	0.19 ± 0.00a	0.17 ± 0.00c	0.18 ± 0.01bc	0.20 ± 0.00a	0.17 ± 0.00c	0.18 ± 0.00b	NS	0.000	0.000

CONV—conventional; ORG—organic; SYS—agricultural system; CV—cultivar.

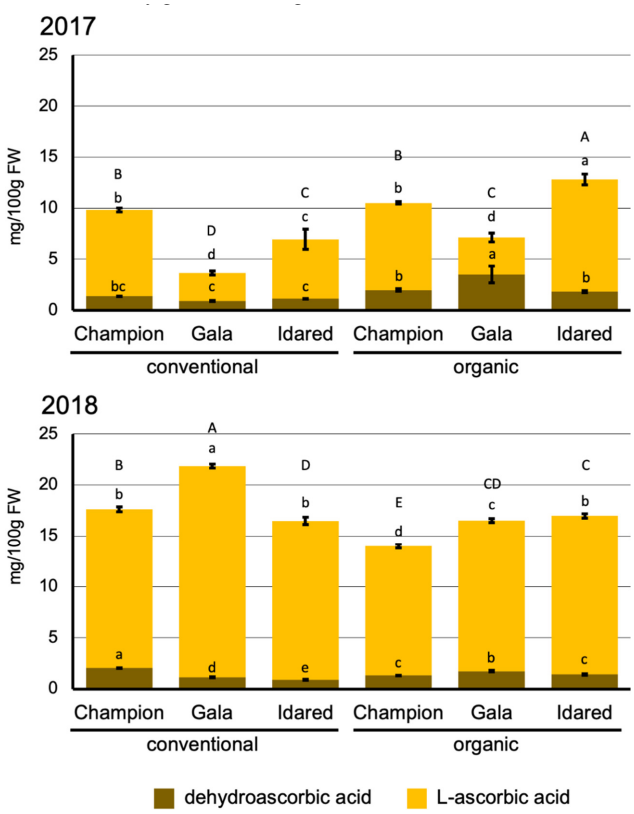

Figure 5. Concentrations (mean and SD) of vitamin C (dehydroascorbic and L-ascorbic acid) in Champion, Gala, and Idared apple fruits collected from organic and conventional orchards in 2017 and 2018 growing seasons. Within each season, bars marked with the same letters are not significantly different at $p < 0.05$ (Lowercase letters: for dehydroascorbic and L-ascorbic acid. Capital letters: for vitamin C (sum)).

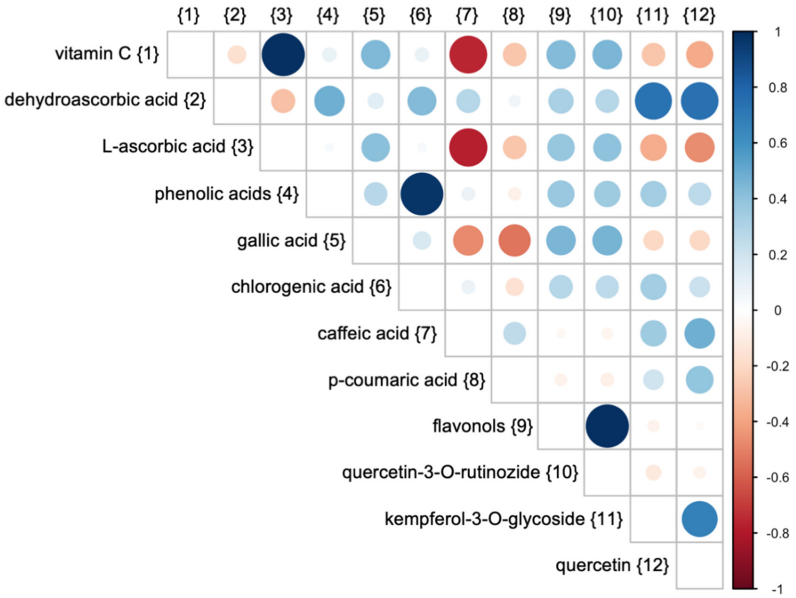

Figure 6. Pearson's correlation between concentrations of individual phytochemicals (phenolics and vitamin C) identified in apple fruit samples. Colour (blue/red) and its intensity correspond to the direction and the strength of the correlation, while the circle size corresponds to its statistical significance (*p*-value).

3. Results and Discussion

Apple is currently the most widely cultivated and one of the most commonly consumed temperate fruits. It is considered as one of the most important fruit sources of antioxidants such as phenolics and vitamin C in the Western diet [3]. After flavanols (catechin and oligomeric procyanidins), known as the major class of apple fruit polyphenols, hydroxycinnamic acids, and flavonols, follow as the second and third most abundant classes, constituting together 3–41% of all apple phenolics [20]. The profiles of these biologically active compounds in fruits and vegetables are known to be impacted by genetic (species, cultivar), environmental, and agricultural factors, including those related to the cultivation practices [24].

The apple fruit samples analyzed within this study contained, on average, 15.41 ± 2.92 mg/100 g f.w. of phenolic acids (mainly chlorogenic acid), 4.46 ± 1.96 mg/100 g f.w. of the selected identified flavonols and 12.85 ± 5.21 mg/100 g f.w. of vitamin C (mainly L-ascorbic acid) (Tables 1–3). The concentrations and profiles of the analyzed phenolic compounds as well as vitamin C in the tested apple samples were generally within the ranges reported by other authors [7,25,26].

The horticultural production system had a significant impact on the concentrations of the majority of the analyzed antioxidants in apples. Organic apples, compared to the conventionally grown ones, were characterized by significantly higher concentrations of phenolic acids (av. >31%) and the analyzed flavonols (av. >66%), with the direction of differences being consistent across apple cultivars and harvest seasons (Table 4, Figures 3 and 4). The identified interactions (SYS × CV and SYS × YR) in case of both groups of phenolic compounds (phenolic acids and flavonols) reflected mainly the variation in the magnitude of organic vs. conventional apples composition differences between cultivars and harvest seasons (Figures 3 and 4, Table 4), i.e., the greatest production system effect on the phenolic acids (sum) concentration in apple fruits harvested in both seasons was observed in the Idared cultivar. The magnitude of the differences in flavonol (sum) concentrations was greater in 2017 compared to the 2018 harvest season. This supports the findings of the recently published meta-analysis on the composition of organic vs. non-organic plant-based foods, which reported that organically cultivated fruits and vegetables are, on average, up to 60% richer in phenolics when compared to the conventionally cultivated ones. However, significant heterogeneity between results was reported for different species, locations, and other conditions [19].

Veberic et al. (2005) [27], in their study on 11 apple cultivars from organic and integrated production in Austria and Slovenia, found higher contents of phenolics in the pulp (but not peels) of organically cultivated apples compared to the fruit coming from integrated production. The authors explained the higher concentrations of phenolic compounds in the pulp of organically grown fruits as a result of increased plant responses to stress in the organic system. Mikulic-Petkovsek et al. (2010) [28] measured the effect of organic and integrated cultivation on the content of phenolics in fruits and leaves of four apple cultivars over a two-year period. The authors underlined that phenolics in fruit may be influenced by many factors such as the fruit type, cultivation, and environmental conditions, growing season, storage environment, and time, and, lastly, by processing and preservation methods. However, in their study, higher levels (10–20%) of total phenolics as well as of each of the analyzed groups of phenolic compounds (hydroxycinnamic acids, flavanols, dihydrochalcones, quercetin) were found in organic vs. integrated apple fruit and leaves. At the same time, Yuri et al. (2012) [29] found no consistent, significant effects of the cultivation management (conventional vs. organic) on polyphenol concentrations and antioxidant activity in three apple cultivars (Gala, Granny Smith, and Fuji) except certain stages of fruit development. In their study, the genotype (cultivar) effects were much more evident. In the review of Kalinowska et al. (2014) [30], the authors also underlined that the content of individual phenolic compounds in the apple fruit strongly depends on apple variety. Jakopic et al. (2012) [31] compared the concentrations of 18 secondary metabolites (hydroxycinnamic acids, flavan-3-ols, dihydrochalcones, and flavonols) in apple fruits from organic, integrated, and two combined systems, and reported that the contents of a majority of identified compounds were highest in the organic system.

The described tendency for higher contents of phenolics in organically grown crops, observed in this study as well as reported by other authors, could be explained by (a) reduced synthesis of secondary metabolites in plants fertilized with easily available mineral nitrogen in the conventional farming systems, and/or (b) increased synthesis of secondary metabolites in plants not protected with synthetic pesticides in the organic systems (increased exposure of plants to stress factors leading to intensive production of phenolics as a defense mechanism) [32,33]. It was previously reported that, in the environments low in easily soluble nitrogen (i.e., organic orchards), plant metabolism shifts towards production of secondary metabolites such as phenolic compounds. In contrast, the use of mineral nitrogen in conventional farming was previously demonstrated to lower phenolic concentrations in fruits and vegetables [34]. In the presented study, the only exception, with no significant organic vs. conventional system-related differences identified in case of phenolic acids (sum), and with higher content of flavonols (sum) in conventional apple fruits, were Champion apples harvested in 2018 (Figures 3 and 4, Table 4).

It is well documented that the phytochemical composition of apple fruit varies greatly between different cultivars of apples, harvest time, or geographic location [4,7]. The significant cultivar and harvest season effect on the concentration of fruit phenolics was also identified in the presented study. A number of significant interactions between the effect of these two factors on phenolics' concentrations in the fruit were also observed, i.e., (1) in the organic system, in the 2017 season, Champion cv. was characterised by the highest, while Idared cv. was characterised by the lowest content of flavonols (sum) among the tested cultivars. In the 2018 harvest season, the contrasting results were reported—the highest flavonols (sum) content in Idared apples, and the lowest in Champion cv.,(2) in the conventional system, in the 2017 season, there were no significant differences in the content of phenolic acids (sum) between the studied cultivars, while, in 2018, Champion was richest in phenolic acids (sum), which was followed by Gala, and then by Idared (Table 4).

Chlorogenic acid was a dominating phenolic acid in the apple fruit, constituting 78–91% of the identified individual acids. This corresponds to the other authors' reports on the hydroxycinnamic acids profile of apples [26]. The described differences in the phenolic acids (sum) content in apples of tested cultivars and production systems in most cases reflected the differences in the chlorogenic acid content (Table 4). In case of other phenolic acids (gallic, caffeic, and *p*-coumaric), various trends in the effects of the production systems and cultivars in the two harvest seasons were observed. Gallic acid concentrations were low and consistent among cultivars in 2017, while, in 2018, a strong interaction between the production system and cultivar was identified. Champion cv. was characterized with the lowest gallic acid content among the three cultivars when grown in the organic system, but it contained the highest concentrations of this compound when grown conventionally (Table 4). Caffeic acid concentrations in the fruits were higher in 2017 when compared to the 2018 growing season (which was consistent for all cultivars in both production systems). However, some interactions between the factors were also identified. While in the organic system, Champion cv. presented the highest contents of caffeic acid among all cultivars in both years. In the conventional system, such a consistent cultivar effect was not confirmed. In 2017 Gala cv. was richest in caffeic acid, but, in 2018, the same cultivar contained the lowest concentrations of this compound among all tested cultivars. In case of *p*-coumaric acid, large differences between harvest seasons were observed in Champion and Idared cv. (with lower contents of *p*-coumaric acid in 2018 compared to 2017) while Gala was characterized by similar (high) contents of this compound in both seasons. Moreover, when comparing apples from organic vs. a conventional system, content of p-coumaric acid was consistently higher in those organically grown (except for Gala cv. harvested in 2017) (Table 4).

Quercetin-3-*O*-rutinozide constituted in most cases nearly 90% of the individual flavonols identified in the tested apple fruits. The described differences in the analyzed flavonols (sum) content in apples reflected, therefore, in most cases, the differences in the quercetin-3-*O*-rutinozide content (Table 4). In case of other identified flavonols (quercetin and kempferol-3-*O*-glycoside), various trends in the effects of the production systems and cultivars in the two harvest seasons were observed (with

significant 2-factor and 3-factor interactions). In case of both compounds, higher concentrations were identified in organic vs. conventional apples during the 2017 harvest season with no such trend observed in 2018 (Table 4).

Vitamin C content in the fruits was strongly dependent on the year-to-year differences in the fruit growing conditions. Higher concentrations of vitamin C were generally found in apples harvested in 2018 (17.23 ± 2.44 mg/100 g f.w.) compared to 2017 (8.47 ± 3.07 mg/100 g f.w.) (Figure 5). Moreover, strong interactions between harvest season, cultivar, and production system were observed in case of the concentrations of this compound, i.e., Gala apples were characterized by the lowest concentrations of L-ascorbic acid in 2017, but were richest in this compound in the following year (especially in the conventional production system). Moreover, while in 2017 Gala apples were richer in vitamin C when grown in the organic system, in 2018, an opposite production system effect was observed in this cultivar with higher concentrations of vitamin C in the conventionally cultivated fruit. Only in case of Idared cultivar, the consistent production system effect across both harvest seasons was observed with higher concentrations of vitamin C found in the organic fruit when compared to the conventionally grown fruit (Figure 5).

L-ascorbic acid was the main vitamin C fraction in the apple fruit, constituting more than 87% of the total vitamin C content. Thus, the described differences in the vitamin C content in apples, in most cases, were reflected in the differences of the L-ascorbic acid content. The highest concentrations of L-ascorbic acid were found in conventional Gala apples harvested in 2018, and the lowest concentrations were found in both organic and conventional Gala apples harvested in 2017. There was an interesting pattern of dehydroascorbic acid concentrations in the apples. In the organic system, the highest concentrations of dehydroascorbic acid were identified in Gala cv., while, in the conventional system, Champion apples were richest in this compound. This trend was consistent in both harvest seasons. Among all tested samples, the highest dehydroascorbic acid contents were found in organic Gala apples harvested in 2017, while the lowest contents of this vitamin C fraction were identified in conventional Gala apples harvested in 2017 and conventional Idared apples harvested in 2018 (Table 4).

The correlation analyses identified a number of significant associations between concentrations of different groups and individual antioxidants in apple fruit samples (Figure 6). The strongest positive correlations were identified between concentrations of dehydroascorbic acid and quercetin ($r = 0.75$, $p = 0.005$), dehydroascorbic acid and kempferol-3-*O*-glycoside ($r = 0.073$, $p = 0.007$), and quercetin and kempferol-3-*O*-glycoside ($r = 0.68$, $p = 0.015$). Significant negative correlation was identified between the concentrations of caffeic acid and L-ascorbic acid ($r = -0.77$, $p = 0.003$) (Figure 6).

4. Conclusions

It is of importance for the consumers to have access to high-quality foods, abundant in health-promoting bioactive compounds. Therefore, looking into the impact of potential quality-modulating factors, such as horticultural practices, on the chemical composition of vegetables and fruits, is of high relevance. Our study demonstrated variation in the content of selected groups of phenolics between apple fruit grown in organic and conventional orchards. Organic apples tested within the study, compared to the conventionally grown ones, were characterized by significantly higher concentrations of phenolic acids and the analyzed flavonols, with the differences being consistent in three apple cultivars and two harvest seasons. The greatest production system effect on the phenolics' concentrations was observed in Idared cultivar. Significant cultivar and season effects on the concentration of the measured phenolic compounds with strong interactions between the two factors were also identified. The consistent production system-related differences were not found in case of vitamin C. Its content in the fruits was strongly dependent on the year-to-year variation in the fruit growing conditions.

These results, which show, in most cases, a favorable composition of organic apples compared to conventional apples of three tested genotypes, and giving insight into the identification of cultivar(s) with the highest quality characteristics, could be of interest for the producers and the consumers

who increasingly search for fruits and vegetables from natural, well-controlled production systems. However, the identified trends should be further confirmed with attention paid to the potential interactions between the plant genotype, horticultural system, and the location-specific growing conditions, to validate the conclusions. The next important step would be to define and to promote the processing and preservation technologies that would assure bringing the healthy attributes of the studied fruits from farm to fork through the processing step.

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