



Article

Impact of Apple Cold Storage on the Physicochemical and Bioactive Quality of Juice

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Abstract

This study compared the quality and bioactive composition of cloudy apple juices produced from four traditional and four conventional apple cultivars immediately after harvest and following cold storage of the fruit at 4 °C for three and six months. Apples were harvested at the ripening stage at the same criteria, stored as whole fruit, and processed into cloudy juice after harvest, three, and six months of storage. Physicochemical parameters and sugar composition were determined, while phenolic compounds were quantified by HPLC-PDA. Antioxidant activity, total phenolic, and flavonoid content were measured spectrophotometrically. All analyses were performed in technical triplicate. The results revealed notable differences between traditional and conventional cultivars. Juices produced from traditional apple cultivars exhibited significantly higher total polyphenol and flavonoid contents than those from conventional cultivars. Significant variations in catechin, myricetin, quercetin, and epigallocatechin levels were also observed among cultivars. The traditional apple cultivar ‘Mašanka’ showed higher concentrations of quercetin (0.09 ± 0.01 µg/mL), chlorogenic acid (486.58 ± 5.48 µg/mL), catechin (8.76 ± 0.54 µg/mL), epicatechin (20.22 ± 0.20 µg/mL), and phloridzin (13.48 ± 0.19 µg/mL) compared to the other cultivars. In contrast, conventional cultivars showed higher concentrations of myricetin and procyanidin B1. Moreover, the content of TA, sucrose, and glucose decreased, whereas pH, fructose, TSS (except for ‘Fuji’ and ‘Granny Smith’) increased. The TFC decreased in traditional apple cultivars, while it increased in conventional cultivars; however, the TFC in conventional cultivars remained lower than in traditional ones. Overall, these findings demonstrate that the cold storage of apples significantly affects juice composition and highlight the advantages of traditional apple cultivars for producing juices with enhanced phenolic content and antioxidant activity.

Keywords: traditional apple cultivars; polyphenols; flavonoids; antioxidant activity



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1. Introduction

Apple juice represents a widely consumed beverage on a global scale, attributable to its desirable organoleptic properties, nutritional value, and reported potential health

benefits [1]. The quality of the juice is influenced by multiple factors, including agricultural practices, cultivar selection, and processing methods [2,3]. Beyond its quality and taste, apple juice is rich in flavonoids, antioxidants, and polyphenols [4]. Recently, interest has grown in comparing apples and apple products from traditional apple cultivars with those from conventional ones, focusing on bioactive composition, taste, and health benefits [5]. Traditional apple cultivars, cultivated for generations and adapted to local environments, are valued for their unique flavor, rich genetic diversity, and high levels of polyphenols and antioxidants [6]. Polyphenols are the predominant bioactive phytochemicals in apples and are classified into five primary groups: flavonols, flavanols, phenolic acids, dihydrochalcones, and anthocyanins [7,8]. Numerous studies have indicated that traditional apple cultivars are characterized by elevated concentrations of bioactive compounds relative to conventional ones. In agreement with prior studies, our investigation revealed that traditional cultivars possessed substantially higher levels of polyphenolic compounds compared with conventional apples [9]. The polyphenolic profile of traditional cultivars was dominated by chlorogenic acid, epicatechin, phloridzin, and procyanidin B1. Additionally, Moressi et al. [10] observed that heritage Italian apple cultivars exhibited significantly higher amounts of total polyphenols and antioxidant activity than conventional ones. For example, traditional apple cultivars ‘Calville White Winter’ and ‘Musò Di Bue’ (360.75 ± 70.87 mg/100 g; 97.32 ± 4.48 mg/100 g) had a higher amount of total polyphenols than conventional ones ‘Golden Delicious’ and ‘Stark Delicious’ (59.51 ± 1.78 mg/100 g; 76.65 ± 2.92 mg/100 g). Moreover, flavanols were the most representative polyphenolic compounds in apples, accounting for between 72% and 96% of the total polyphenolic content. Similarly, Preti et al. [11] concluded in their study that the flesh and peel of Italian traditional apple cultivars had a higher content of antioxidant compounds than conventional ones, with flavonols such as rutin and quercetin presented only in traditional apple cultivars. Phloridzin, phloretin, and dihydrochalcones were mostly present in the apple peel. The highest content of phloridzin in the peel was detected in the traditional apple cultivar ‘Di Corone’ (13.53 ± 0.67 mg/100 g) and in ‘Dal Cordon’ (6.0 ± 1.08 mg/100 g) for phloretin. The total polyphenol content in traditional apple cultivars ranged from 51.38 to 146.63 mg/100 g and in conventional ones from 43.45 to 74.55 mg/100 g, highlighting that the average polyphenol content in the peel of traditional apples is almost three times higher than in the pulp and four times higher in conventional ones. This evidence supports the hypothesis that breeding selection has focused on improving the organoleptic properties of the pulp, as it is the most consumed part of the apple, leading to a reduction in polyphenol content [12]. The study conducted by Veberic et al. [13] highlights the significantly higher amounts of phenolic compounds in the traditional Slovenian apple cultivar compared to conventional ones, with procyanidin B2, chlorogenic acid, and epicatechin being the most abundant. Moreover, they highlight that plants cultivated under organic conditions are frequently exposed to various environmental stresses, which may stimulate polyphenol synthesis. Apple juice has attracted attention for its antioxidant properties, largely due to polyphenols and flavonoids linked to reduced risks of chronic conditions [14,15]. Beyond its nutritional and health-related properties, the composition of sugars and organic acids in apple juice plays a key role in its sensory characteristics and consumer preference. Akagic et al. [16] reported that traditional apple cultivars possess higher concentrations of organic acids, sugar, and total polyphenols relative to conventional ones. Differences in the composition of sugars and organic acids can influence the flavor, aroma, and overall quality of apple juice, potentially making traditional apple cultivars more appealing to consumers seeking varied and distinctive flavors [17,18]. Furthermore, the diversity of traditional apple cultivars can lead to a wide range of apple juices, each offering unique

sensory and nutritional characteristics. Using traditional cultivars for juice production also contributes to sustainability and supports the preservation of biodiversity.

The aim of this study was to evaluate the quality of apple juices produced from traditional and conventional apple cultivars, focusing on total polyphenols, flavonoids, sugar content, antioxidant activity, pH, TSS (°Brix), and total acids. Finally, the study aims to test the hypothesis that traditional apple cultivars have higher contents of bioactive compounds compared to conventional ones and to evaluate their potential for diversified juice production, as well as to determine how storage under certain conditions affects the chemical composition and overall quality of the resulting juice

2. Materials and Methods

2.1. Chemicals and Reagents

Total polyphenols were quantified using the Folin-Ciocalteu reagent (Sigma-Aldrich, Chemie Check GmbH, Steinheim, Germany) and sodium carbonate (Na_2CO_3 ; GRAM-MOL d.o.o., Zagreb, Croatia). Antioxidant capacity was assessed by DPPH, ABTS, and Trolox assays (Sigma-Aldrich, Chemie Check GmbH, Steinheim, Germany), with potassium persulfate supplied by Fischer Chemical (Loughborough, UK). Total flavonoids were determined using aluminum chloride (98%, extra pure; Alfa Aesar, Ward Hill, MA, USA, SAD), sodium nitrite (GRAM-MOL d.o.o., Zagreb, Croatia), and sodium hydroxide (T.T.T d.o.o., Sveta Nedelja, Croatia). Methanol (MS grade) was obtained by Scharlaub (Chemie S.A., Barcelona, Spain), and ultrapure water was produced using a Mili-Q gradient system (Millipore, Bedford, MA, USA). Individual polyphenol standards (2000–8000 $\mu\text{g}/\text{mL}$ in methanol), including *trans*-ferulic acid, chlorogenic acid, catechin, epigallocatechin, caffeic acid, procyanidin A2, *p*-coumaric acid, phloridzin, epicatechin, myricetin, quercetin-3-D-glucoside, procyanidin B1, quercetin, and procyanidin B2, as well as sucrose, glucose, and fructose for sugar calibration, were purchased from Sigma-Aldrich (Chemie Check GmbH, Steinheim, Germany). Quantification of individual polyphenols and sugars was performed using external calibration curves constructed from at least five concentration levels of each standard.

2.2. Plant Material

Traditional (heritage) apple cultivars ‘Ilzer Rosenapfel’ and ‘Ivanlija’ were harvested in Šašince, Experimental site of the Department of Fruit Growing, University of Zagreb, Faculty of Agriculture, Croatia (45°85′00.3″ N, 16°17′75.2″ E), while ‘Austrougarka’ and ‘Mašanka’ were collected in Perenci, Požega, OPG (family farm business) Katić (45°23′45.0″ N, 17°34′06.3″ E). The conventional apple cultivars ‘Granny Smith’, ‘Fuji’, ‘Jonagold’, and ‘Golden Delicious’ were obtained from an orchard located in Novaki Bistranski, Donja Bistrica, Agro-Car, Croatia (45°54′57.0″ N 15°52′56.0″ E). All apples were harvested during September 2023 at the ripening stage. The orchards are located in a temperate continental climate zone, and soil characteristics varied among orchards: loamy soil with low humus and phosphorus content and good nitrogen and potassium availability (Novaki Bistranski); soil with acidic reaction, low humus and phosphorus content and adequate supply of nitrogen and potassium (Perenci); and loamy alkaline soil with low humus and adequate supply of phosphorus and potassium (Šašince). The identity of all cultivars was confirmed by a pomologist, and the classification was further validated through genotyping using 12 SSR markers [19]. Both traditional and conventional apples were stored in a controlled atmosphere at 4 °C, with a relative humidity of approximately 90–95%, natural ventilation, and without packaging (stored in apple crates). Apples were stored for 0, 3, and 6 months, after which juice production was performed. No postharvest fungicide treatments were applied. Cloudy apple juice was produced immediately after

harvest and after 3 and 6 months of cold storage. For each cultivar and storage period, juice was prepared from approximately 2 kg of fruit. Three technical replicates were performed on each individual juice sample to assess the repeatability of the measurements. Juice was produced using a laboratory juicer by crushing and pressing the fruit. From each batch, 500 mL of freshly obtained cloudy juice was divided into two portions: One portion (250 mL) was pasteurized at 80 °C for two minutes, sealed in appropriate containers, and used for the determination of physicochemical parameters. The other portion (250 mL) was kept unpasteurized and used immediately for the determination of individual polyphenols, flavonoids, antioxidant activity, and sugar composition. For the analysis of individual polyphenols and sugar composition, unpasteurized juice was filtered through a 0.22 µm PTFE filter.

2.3. Determination of Physico-Chemical Composition

Total acidity was determined by titration with 0.1 M NaOH using phenolphthalein as an indicator and expressed as g malic acid per 100 mL of juice. Sample pH was measured with a Mettler Toledo pH meter (Columbus, OH, USA). The soluble dry matter (TSS) was measured using the Abbe refractometer and reported as °Brix.

2.4. Determination of Sugar

Carbohydrates were analyzed by HPLC-RI using a Zorbax NH₂ column (Agilent, Santa Clara, CA, USA, 4.6 × 12.5 mm, particle size 5 µm, 400 bar pressure limit). The mobile phase consisted of acetonitrile and water (70:30, v/v) at a flow rate of 1 mL/min, with a 10 µL injection volume. The column and the refractive index detector were maintained at 45 °C. Glucose, fructose, and sucrose (≥99% purity; Sigma-Aldrich, Steinheim, Germany) were identified by a comparison of their retention times with those of external reference standards. The total run time was 20 min per sample. Retention times were approximately 6.057 for fructose, 6.578 for glucose, and 7.929 for sucrose, results were expressed as g/L juice.

2.5. Total Polyphenol Content Determination

The total polyphenol content was determined using the Folin-Ciocalteu method. The color change of the reaction mixture was measured after 2 h at room temperature using a spectrophotometer (Perkin Elmer, LAMBDATM365, Waltham, MA, USA) at 765 nm. Measurements were performed in triplicate, and results were calculated using an external calibration curve constructed with gallic acid over the concentration range of 0–150 mg/L ($R^2 = 0.9995$), with corresponding absorbance values ranging from 0 to 1.205, and expressed as mg/L.

2.6. Determination of Polyphenol Profile

The polyphenol profile of each sample was determined by HPLC using a Shimadzu system equipped with a photodiode array detector (PDA). Separation was achieved on an Inertsil ODS-3V column (GL Sciences; 250 × 4.6 mm, 5 µm particle size). Data were processed with LabSolutions software (Release 5.52). The mobile phase consisted of 1% formic acid in water (purity 98–100%; Scharlau Chemie, Sentmenat, Spain) as solvent A and 1% formic acid in methanol (purity ≥ 99.9%; J. T. Baker, Deventer, The Netherlands) as solvent B. The injection volume was 10 µL, the column and detector temperatures were maintained at 50 °C, and the flow rate was 0.8 mL/min using gradient elution. Detection wavelengths were 280, 320, 360, and 520 nm, depending on compound class. Identification was based on comparing their retention times and UV–Vis spectra with those of authentic standards, and the total run time was 35 min per sample. All standards used for calibration

had a purity of ≥ 98 –99% purity. Quantification was performed using external calibration curves of individual standards ($R^2 > 0.99$), and results were expressed as $\mu\text{g/mL}$.

2.7. Determination of Flavonoids

Total flavonoids were measured using a modified colorimetric method based on Makris et al. [20] involving the sequential reaction of diluted juice with NaNO_2 , AlCl_3 , and Na_2CO_3 . The absorbance was measured at 510 nm after color development, and analyses were performed in triplicate. Flavonoid content was calculated by interpolating the absorbance values against a catechin calibration curve and expressed as mg CE/L of apple juice.

2.8. Determination of Antioxidant Activity

The $\text{ABTS}^{\bullet+}$ scavenging activity was determined using a modified method of Arnao et al. [21]. For the assay, 0.2 mL of sample was mixed with 3.2 mL of ABTS solution, and the absorbance was measured at 734 nm after 1 h 35 min. Results were expressed as mmol Trolox equivalents per liter of sample (mmol TE/L) ($R^2 = 0.9904$).

The DPPH $\bullet$ scavenging activity assay was determined using a modified method of Brand-Williams et al. [22]. The reaction mixture contained 0.2 mL sample, 2 mL of methanol, and 1 mL of a 0.5 mM DPPH solution in ethanol, followed by absorbance recording at 517 nm. Results were expressed as mmol TE/L ($R^2 = 0.9915$). Samples exceeding the linear calibration range were diluted prior to analysis.

2.9. Statistical Analysis

Results are expressed as the mean value $\pm$ SD (standard deviation) based on three independent replicates. Because the normality and homogeneity of variance were not satisfied, nonparametric tests, namely the Mann–Whitney U test and Kruskal–Wallis ANOVA, were applied to compare traditional and conventional apple cultivars. All statistical analyses were performed using Statistica version 13.5 (TIBCO Software INC., Palo Alto, CA, USA), and differences were considered statistically significant at $p < 0.05$ (Table S1 in Supplementary Materials).

3. Results and Discussion

In this study, the contents of total acids, polyphenols, flavonoids, pH, TSS ($^\circ\text{Brix}$), antioxidant activity, and individual polyphenols were investigated and compared in juices obtained from the examined apple cultivars during six months of cold storage. Three of the most important parameters affecting the sensory quality, shelf life, and safety of apple juice are total acids, pH, and TSS ($^\circ\text{Brix}$). Table 1 presents the physicochemical characteristics of juices obtained from traditional and conventional apple cultivars. Total acidity reflects the compositional balance of the juice and is a key factor influencing both flavor development and product stability. In the present study, total acid content, expressed as malic acid, ranged from 0.03 to 0.17 g/100 mL. These values are consistent with previously published data, which report total acidity in apple juice between 0.07 and 2.5 g/100 mL, expressed as malic acid [5,23,24]. Variations in total acidity observed during storage is primarily attributed to metabolic processes occurring in the fruit after harvest. Organic acids, particularly malic acid, are gradually metabolized through respiration, which can lead to a decrease in total acidity over time. Additionally, changes in cell structure and membrane permeability during storage can influence the extractability of organic acids into the juice [25]. The results of the pH value ranged from 3.29 (‘Mařanka’) to 4.34 (‘Golden Delicious’). Variations in pH within the same cultivar over different storage periods indicate that storage duration significantly affects apple juice acidity. These changes are closely related to the degradation of organic acids and ongoing biochemical processes during

storage. Moreover, the acidity level of the juice plays a crucial role in its taste, stability, and microbial safety. Our findings are consistent with similar studies that reported that the pH value of apple juice ranges from 3.37 to 4.24 [26,27]. Our results, regarding the proportion of TSS ($^{\circ}$ Brix), showed that it ranged from 13.80 $^{\circ}$ Brix ('Jonagold') to 23.20 $^{\circ}$ Brix ('Mašanka').

Table 1. Physicochemical composition of juices produced from traditional and conventional apple cultivars during cold storage.

Cultivars	pH			TA (g/100 mL as Malic Acid)			TSS ($^{\circ}$ Brix)		
	0	3	6	0	3	6	0	3	6
Storage time									
'Ilzer Rosenapfel'	3.41 $\pm$ 0.01 _{a,b}	3.58 $\pm$ 0.04 _{a,b}	3.77 $\pm$ 0.02 _b	0.08 $\pm$ 0.01 _c	0.08 $\pm$ 0.00 _d	0.07 $\pm$ 0.01 _c	15.90 $\pm$ 0.04 _c	16.20 $\pm$ 0.06 _c	17.10 $\pm$ 0.05 _c
'Ivanlija'	3.89 $\pm$ 0.00 _d	3.99 $\pm$ 0.01 _c	4.31 $\pm$ 0.00 _d	0.04 $\pm$ 0.00 _a	0.04 $\pm$ 0.01 _b	0.03 $\pm$ 0.00 _a	14.30 $\pm$ 0.07 _b	15.20 $\pm$ 0.02 _b	15.00 $\pm$ 0.03 _b
'Mašanka'	3.40 $\pm$ 0.02 _a	3.47 $\pm$ 0.00 _a	3.29 $\pm$ 0.01 _a	0.17 $\pm$ 0.02 _f	0.13 $\pm$ 0.03 _g	0.12 $\pm$ 0.02 _d	19.20 $\pm$ 0.04 _f	22.10 $\pm$ 0.07 _e	23.20 $\pm$ 0.09 _d
'Austrougarka'	3.51 $\pm$ 0.01 _b	3.72 $\pm$ 0.00 _b	3.66 $\pm$ 0.01 _b	0.12 $\pm$ 0.00 _d	0.09 $\pm$ 0.02 _e	0.07 $\pm$ 0.00 _c	16.50 $\pm$ 0.02 _d	16.60 $\pm$ 0.03 _c	17.20 $\pm$ 0.01 _c
'Jonagold'	3.70 $\pm$ 0.01 _c	4.10 $\pm$ 0.02 _d	4.09 $\pm$ 0.01 _c	0.05 $\pm$ 0.00 _b	0.03 $\pm$ 0.00 _a	0.03 $\pm$ 0.00 _a	13.80 $\pm$ 0.05 _a	14.80 $\pm$ 0.06 _a	14.40 $\pm$ 0.03 _a
'Golden Delicious'	3.82 $\pm$ 0.00 _{c,d}	4.22 $\pm$ 0.00 _d	4.34 $\pm$ 0.04 _d	0.05 $\pm$ 0.00 _b	0.03 $\pm$ 0.00 _a	0.03 $\pm$ 0.01 _a	15.70 $\pm$ 0.07 _c	16.60 $\pm$ 0.04 _c	15.90 $\pm$ 0.06 _b
'Fuji'	3.71 $\pm$ 0.02 _c	3.94 $\pm$ 0.01 _c	4.14 $\pm$ 0.03 _c	0.08 $\pm$ 0.02 _c	0.07 $\pm$ 0.01 _c	0.05 $\pm$ 0.00 _b	18.20 $\pm$ 0.03 _e	20.10 $\pm$ 0.09 _d	17.40 $\pm$ 0.05 _c
'Granny Smith'	3.30 $\pm$ 0.02 _a	3.42 $\pm$ 0.00 _a	3.50 $\pm$ 0.00 _{a,b}	0.14 $\pm$ 0.01 _e	0.12 $\pm$ 0.03 _f	0.12 $\pm$ 0.02 _d	14.90 $\pm$ 0.01 _b	15.20 $\pm$ 0.04 _b	14.00 $\pm$ 0.03 _a

TSS—soluble dry matter; TA—total acids. Mean $\pm$ SD based on three juice samples ($n = 3$); within the same column, means followed by different letters are significantly different at $p \leq 0.05$.

Notably, the results also showed that the soluble dry matter content in apple juice increased proportionally with the duration of apple storage. This increase is mainly associated with the conversion of starch into soluble sugars during postharvest ripening, as well as with water loss through transpiration, which leads to the concentration of soluble compounds. Soluble dry matter provides important information about the amount of solids in the juice and is closely related to its sweetness and overall sensory quality. This finding agrees with our previous study that reported the TSS content to range from 14.1 to 18.5 $^{\circ}$ Brix [5].

Table 2 summarizes the sugar profile of juices obtained from traditional and conventional apple cultivars during six months of cold storage. Apples primarily contain sucrose, fructose, glucose, and sorbitol as their main soluble sugars [28]. The results revealed statistically significant differences ($p \leq 0.05$) in the relative proportions of glucose, sucrose, and fructose among the examined cultivars (Table S2), indicating a strong influence of genetic factors on sugar composition. The proportion of sucrose in the juice samples ranged from 13.77 $\pm$ 1.60 g/L ('Ivanlija', six months) to 138.83 $\pm$ 2.18 g/L ('Mašanka', three months). The proportion of glucose in the juice samples ranged from 6.39 $\pm$ 0.20 g/L ('Ivanlija', six months) to 66.04 $\pm$ 1.89 g/L ('Fuji', three months), while fructose content ranged from 23.04 $\pm$ 0.51 g/L ('Ivanlija', three months) to 125.02 $\pm$ 2.28 g/L ('Mašanka', six months). The content of soluble sugars differs among cultivars and depends on several factors, including sun exposure, light intensity, temperature conditions ripening stage, and cultivar specific metabolic characteristics. During apple ripening and storage, starch is enzymatically hydrolyzed into soluble sugars through the activity of enzymes such as amylases, while sucrose is further cleaved into glucose and fructose by invertase [29,30]. In addition, sugars are continuously utilized as substrates in respiration, which can contribute to a gradual decrease in total sugar content during prolonged storage. Our results showed that during storage, the content of sucrose and glucose decreased, while the fructose content increased. The reduction in sucrose concentration can be attributed mainly to its hydrolysis into the constituent monosaccharides, whereas the decrease in glucose may be related to its preferential utilization in respiratory metabolism. The relative increase in fructose

content may result from both the sucrose hydrolysis and differential metabolic utilization of individual sugars, as fructose is often metabolized more slowly than glucose during storage. These findings are consistent with similar studies that reported a higher amount of fructose and a lower amount of sucrose content in examined apple cultivars [5,31,32]. Additionally, fructose is the preferred sugar in apple juice due to its sweeter taste. Therefore, the fructose-to-glucose ratio represents an important indicator of sweetness and overall flavor quality. The results indicate that the cultivars with the highest fructose-to-glucose ratio, including ‘Mašanka’ 12.19 and ‘Ivanlija’ 9.85, are expected to produce juices with a sweeter taste compared to the other examined apple cultivars.

Table 2. Comparison of sugar profile in juices produced from traditional and conventional apple cultivars during cold storage.

Cultivars	Sucrose [g/L]			Glucose [g/L]			Fructose [g/L]		
Storage time	0	3	6	0	3	6	0	3	6
‘Ilzer Rosenapfel’	67.22 ± 1.08 _a	45.32 ± 0.61 _a	48.86 ± 1.28 _d	26.68 ± 0.33 _b	26.53 ± 0.20 _a	9.25 ± 0.76 _a	63.22 ± 1.23 _c	38.51 ± 2.97 _b	72.50 ± 2.75 _b
‘Ivanlija’	82.40 ± 0.19 _c	96.03 ± 0.08 _c	13.77 ± 1.60 _a	21.30 ± 0.02 _a	24.44 ± 0.03 _a	6.39 ± 0.20 _a	24.84 ± 0.88 _a	23.04 ± 0.51 _a	62.95 ± 1.26 _a
‘Mašanka’	92.71 ± 1.92 _d	138.83 ± 2.18 _e	76.70 ± 1.40 _e	22.70 ± 0.32 _a	36.82 ± 0.27 _c	10.26 ± 0.40 _a	82.68 ± 1.43 _d	78.48 ± 2.59 _d	125.02 ± 2.28 _e
‘Austrougarka’	81.30 ± 0.05 _c	83.01 ± 0.45 _b	48.07 ± 0.48 _d	22.36 ± 0.04 _{a,b}	29.25 ± 0.60 _b	9.55 ± 0.29 _a	61.49 ± 0.18 _c	50.39 ± 0.06 _c	82.66 ± 0.23 _c
‘Jonagold’	75.04 ± 0.87 _b	83.94 ± 1.86 _b	30.21 ± 1.46 _b	25.71 ± 1.30 _b	27.89 ± 0.51 _{a,b}	17.47 ± 0.30 _b	48.13 ± 1.92 _b	43.23 ± 0.42 _b	73.27 ± 0.50 _b
‘Golden Delicious’	88.72 ± 0.56 _{c,d}	112.99 ± 4.38 _d	29.07 ± 0.88 _b	29.89 ± 0.05 _{b,c}	44.25 ± 1.38 _d	20.62 ± 0.22 _{b,c}	48.07 ± 0.66 _b	41.87 ± 4.79 _b	78.19 ± 0.83 _{b,c}
‘Fuji’	91.44 ± 0.17 _d	120.85 ± 3.84 _d	30.76 ± 1.21 _b	50.62 ± 0.55 _d	66.04 ± 1.89 _f	30.23 ± 0.19 _d	49.85 ± 1.61 _b	37.74 ± 0.29 _b	92.43 ± 0.63 _d
‘Granny Smith’	65.87 ± 1.08 _a	90.84 ± 0.45 _c	38.81 ± 3.80 _c	31.34 ± 0.57 _c	54.48 ± 0.35 _e	25.22 ± 0.40 _c	46.19 ± 1.92 _b	41.73 ± 0.58 _b	72.33 ± 1.16 _b

Mean ± SD based on three juice samples ($n = 3$); within the same column, means followed by different letters are significantly different at $p \leq 0.05$.

Furthermore, the contents of total polyphenols (TPC) and total flavonoids (TFC) were determined in the examined apple juices (Table 3). The highest TPC values after harvest were observed in the traditional cultivar ‘Mašanka’ (610.92 ± 18.76 mg GAE/L, after harvest), followed by ‘Austrougarka’ (541.75 ± 15.21 mg GAE/L, after harvest). During storage, a decreasing trend in both TPC and TFC was observed across all cultivars. After six months of cold storage, the highest TPC was still recorded in the traditional apple cultivar ‘Mašanka’ (163.40 ± 1.18 mg GAE/L), indicating a relatively high retention of polyphenolic compounds compared to other cultivars. In contrast, the lowest TPC after harvest was found in the conventional apple cultivar ‘Jonagold’ (226.75 ± 13.92 mg GAE/L), followed by ‘Granny Smith’ (257.58 ± 5.77 mg GAE/L), while after six months of storage, the lowest TPC was recorded in ‘Ivanlija’ (21.36 ± 3.32 mg GAE/L). A similar trend was observed for TFC. The highest TFC after harvest was measured in ‘Mašanka’ (154.69 ± 2.27 mg CE/L), followed by ‘Austrougarka’ (107.30 ± 6.46 mg CE/L), whereas the lowest value was detected in ‘Golden Delicious’ (23.36 ± 0.81 mg CE/L). After six months of storage, the highest TFC was found in ‘Fuji’ (65.02 ± 4.90 mg CE/L), while the lowest value was recorded in ‘Mašanka’ (10.82 ± 2.55 mg CE/L). The observed decrease in TPC and TFC during storage can be attributed to several biochemical and physicochemical processes occurring in apple tissue after harvest. Polyphenolic compounds are susceptible to enzymatic oxidation catalyzed by polyphenol oxidase and peroxidase, leading to the formation of quinones and subsequent polymerization into soluble brown pigments. Additionally, prolonged storage may promote the degradation of phenolic compounds due to oxidative reactions, as well as their binding to cell wall components, which reduces their extractability into juice [33,34]. These results indicate that juices from traditional apple cultivars contained significantly higher amounts of total phenolics (TPC) and total flavonoids (TFC) compared with those from conventional cultivars. In contrast, conventional apples are usually grown

under intensive agricultural systems, which may lead to lower levels of these bioactive compounds [5]. The range observed for TPC (21.36 ± 3.32 to 610.92 ± 18.76 mg GAE/L) and TFC (10.82 ± 2.55 to 154.69 ± 2.27 mg CE/L) are consistent with values reported in earlier studies [9,35–37]. Additionally, the results showed that cultivars with higher TPC values also tended to exhibit higher TFC values, and vice versa. This positive correlation between TPC and TFC has been reported in previous research [38]. The observed relationship between TPC and TFC can be explained by the fact that flavonoids are a subclass of polyphenols.

Table 3. Total polyphenol and flavonoid levels in juices obtained from traditional and conventional apple cultivars throughout cold storage.

Cultivars	TPC (mg GAE/L)			TFC (mg CE/L)		
	0	3	6	0	3	6
Storage time						
‘Ilzer Rosenapfel’	400.08 ± 11.81^c	58.91 ± 17.95^a	38.41 ± 4.11^b	60.23 ± 1.00^c	43.49 ± 12.58^c	27.37 ± 3.59^b
‘Ivanlija’	315.92 ± 5.20^b	54.46 ± 8.90^a	21.36 ± 3.32^a	70.33 ± 1.57^c	30.55 ± 2.45^b	39.92 ± 4.90^c
‘Mašanka’	610.92 ± 18.76^e	283.18 ± 10.81^d	163.40 ± 1.18^f	154.69 ± 2.27^e	48.98 ± 6.04^{cd}	10.82 ± 2.55^a
‘Austrougarka’	541.75 ± 15.21^d	215.66 ± 14.42^c	23.67 ± 4.24^a	107.30 ± 6.46^d	106.63 ± 5.80^e	14.04 ± 9.51^a
‘Jonagold’	226.75 ± 13.92^a	124.03 ± 5.63^b	43.03 ± 0.59^c	14.53 ± 0.62^a	46.24 ± 27.52^c	22.67 ± 8.91^b
‘Golden Delicious’	257.58 ± 5.77^a	141.98 ± 15.22^b	44.18 ± 3.78^c	23.36 ± 0.81^{ab}	8.59 ± 4.24^a	42.27 ± 8.91^c
‘Fuji’	309.25 ± 15.61^b	196.17 ± 3.98^c	65.08 ± 8.74^d	31.97 ± 1.08^b	54.47 ± 27.57^d	65.02 ± 4.90^d
‘Granny Smith’	319.25 ± 12.99^b	199.08 ± 8.01^c	97.78 ± 6.57^e	33.30 ± 2.93^b	$37.22 \pm 2.45^{b,c}$	39.92 ± 5.92^c

TPC—total polyphenol content; TFC—total flavonoid content. Data are presented as mean $\pm$ SD based on three juice samples ($n = 3$). Within each column, means marked with different letters are significantly different at $p \leq 0.05$.

Procyanidins, which represent the predominant class of polyphenolic compounds in apples, were detected in all analyzed samples (Table 4). Statistical analysis showed significant differences ($p \leq 0.05$) in the contents of procyanidin A2, B1, and B2 among the examined apple cultivars (Table S2), indicating a strong cultivar-dependent influence on procyanidin composition. After harvest, the highest content of procyanidin A2 was observed in ‘Golden Delicious’ (2.18 ± 0.08 $\mu\text{g/mL}$), while the lowest was found in ‘Jonagold’ (0.11 ± 0.04 $\mu\text{g/mL}$). For procyanidin B1, the highest level was measured in ‘Granny Smith’ (197.34 ± 3.52 $\mu\text{g/mL}$), whereas the lowest content was recorded in ‘Ivanlija’. Similarly, ‘Mašanka’ exhibited the highest content of procyanidin B2 (159.92 ± 1.76 $\mu\text{g/mL}$), while ‘Ivanlija’ showed the lowest level (0.81 ± 0.17 $\mu\text{g/mL}$). During storage, the content of procyanidin B1 and B2 generally decreased across most cultivars, where procyanidin A2 exhibited cultivar-dependent variations, with both increases and decreases observed. Similar trends have been reported in previous studies, where procyanidin concentrations tended to decline during prolonged storage [39]. These changes can be attributed to several biochemical processes occurring postharvest. Procyanidins are susceptible to enzymatic oxidation and polymerization reactions catalyzed by polyphenol oxidase and peroxidase, leading to the formation of high-molecular-weight compounds that are less extractable in juice. In addition, oxidative degradation and participation in browning reactions contribute to the reduction in measurable procyanidin content during storage [40,41]. Structural changes in cell walls and membranes during storage may also affect the release and extractability of these compounds into the juice matrix. After six months of cold storage, ‘Mašanka’ had the highest procyanidin A2 level (7.25 ± 0.76 $\mu\text{g/mL}$), while the lowest was detected in ‘Fuji’ (0.11 ± 0.00 $\mu\text{g/mL}$). For procyanidins B1 and B2, ‘Granny Smith’ had the highest levels (161.14 ± 35.81 $\mu\text{g/mL}$; 154.11 ± 3.84 $\mu\text{g/mL}$), whereas ‘Ivanlija’ exhibited the lowest values. Overall, traditional apple cultivars tended to show higher procyanidin contents compared to conventional cultivars, which is consistent with previous findings [9,42]. Statistical analysis also revealed significant differences ($p \leq 0.05$) in flavonol contents, including myricetin and quercetin, among the examined cultivars (Table 5, Table S2). Flavonols in apples are predominantly present in the form of quercetin glycosides [42]. After harvest,

the highest myricetin content was observed in ‘Jonagold’ ($0.60 \pm 0.01 \mu\text{g/mL}$), while the lowest was detected in ‘Fuji’ ($0.02 \pm 0.00 \mu\text{g/mL}$). The highest content of quercetin-3-D-glucoside was measured in ‘Austrougarka’ ($1.24 \pm 0.01 \mu\text{g/mL}$), whereas the lowest was found in ‘Jonagold’ ($0.14 \pm 0.00 \mu\text{g/mL}$). The highest quercetin content was measured in ‘Mašanka’ ($0.09 \pm 0.01 \mu\text{g/mL}$). A general decline in flavonol content was observed during storage, which is consistent with previous studies [5,9]. This decrease may be explained by oxidative degradation, enzymatic activity, and interactions with other cellular components during storage. Moreover, flavonols may bind to macromolecules such as proteins and polysaccharides, reducing their extractability into juice. After six months of storage, ‘Mašanka’ retained the highest levels of myricetin and quercetin-3-D-glucoside ($0.41 \pm 0.01 \mu\text{g/mL}$; $0.72 \pm 0.01 \mu\text{g/mL}$), while the lowest levels were observed in ‘Austrougarka’. For quercetin, the lowest level was detected in most juices except those obtained from ‘Ilzer Rosenapfel’, ‘Mašanka’, and ‘Granny Smith’. Variations in quercetin content may be associated with differences in compound stability, extractability, and susceptibility to degradation during storage and juice processing. Previous studies have reported a significant decrease in quercetin concentration in apple juice after pasteurization, primarily due to thermal degradation [43]. It is important to note that flavonols benefit human health through their antioxidant, antimicrobial, anti-inflammatory, and anticancer properties [10,44]. Therefore, the higher flavonol levels found in traditional cultivars such as ‘Mašanka’ may enhance their nutritional value and health benefits compared to conventional varieties.

Table 4. The procyanidin content ($\mu\text{g/mL}$) in juices produced from traditional and conventional apple cultivars during cold storage.

Cultivars	Procyanidin A2 ($\mu\text{g/mL}$)			Procyanidin B1 ($\mu\text{g/mL}$)			Procyanidin B2 ($\mu\text{g/mL}$)		
Storage time	0	3	6	0	3	6	0	3	6
‘Ilzer Rosenapfel’	0.34 ± 0.09 b,c	0.51 ± 0.04 d	2.31 ± 0.07 d	105.46 ± 9.84 f	4.47 ± 0.84 b	5.32 ± 1.85 b	126.21 ± 2.25 e	0.00 ± 0.00 a	3.02 ± 0.16 b
‘Ivanlija’	0.63 ± 0.05 d	0.20 ± 0.02 b	0.39 ± 0.02 b	0.00 ± 0.00 a	2.40 ± 1.28 a	0.00 ± 0.00 a	0.81 ± 0.17 a	0.00 ± 0.00 a	0.00 ± 0.00 a
‘Mašanka’	0.93 ± 0.20 e	0.76 ± 0.04 f	7.25 ± 0.76 e	167.63 ± 3.86 g	80.52 ± 1.81 f	25.01 ± 6.30 e	159.92 ± 1.76 g	43.53 ± 5.08 d	67.50 ± 10.39 c
‘Austrougarka’	0.48 ± 0.03 c	0.59 ± 0.31 e	0.39 ± 0.01 b	20.79 ± 3.48 d	30.12 ± 1.81 d,e	2.50 ± 0.28 b	150.42 ± 0.90 f	42.24 ± 3.60 d	1.92 ± 0.61 b
‘Jonagold’	0.11 ± 0.04 a	2.31 ± 0.02 s	2.51 ± 0.10 d	3.00 ± 0.86 b	19.64 ± 1.48 c	0.00 ± 0.00 a	6.74 ± 0.08 b	34.97 ± 2.47 c	1.35 ± 0.04 b
‘Golden Delicious’	2.18 ± 0.08 f	0.26 ± 0.00 b	2.75 ± 0.26 d	10.37 ± 1.80 c	29.83 ± 0.65 d	15.36 ± 0.93 d	7.38 ± 0.11 b	41.48 ± 1.10 d	5.30 ± 0.50 b
‘Fuji’	0.18 ± 0.05 a	3.22 ± 0.61 h	0.11 ± 0.00 a	36.16 ± 5.20 e	35.52 ± 5.28 e	13.15 ± 0.60 d	37.80 ± 0.46 c	24.64 ± 3.66 b	0.72 ± 0.07 a
‘Granny Smith’	0.40 ± 0.01 c	0.10 ± 0.01 a	1.55 ± 0.21 c	197.34 ± 3.52 h	216.76 ± 22.38 s	161.14 ± 35.81 f	88.01 ± 0.56 e	168.12 ± 8.63 e	154.11 ± 3.84 d

Mean $\pm$ SD based on three juice samples ($n = 3$). Within the same column, means followed by different letters are significantly different at $p \leq 0.05$.

Table 5. The concentration of flavonols ($\mu\text{g/mL}$) in juices derived from traditional and conventional apple cultivars during cold storage.

Cultivars	Myricetin ($\mu\text{g/mL}$)			Quercetin-3-D-Glucoside ($\mu\text{g/mL}$)			Quercetin ($\mu\text{g/mL}$)		
Storage time	0	3	6	0	3	6	0	3	6
‘Ilzer Rosenapfel’	0.15 ± 0.00 c	0.02 ± 0.00 a	0.02 ± 0.00 a	0.45 ± 0.01 c	0.00 ± 0.00 a	0.08 ± 0.01 b	0.06 ± 0.00 b	0.05 ± 0.00 b	0.05 ± 0.01 b
‘Ivanlija’	0.10 ± 0.00 b	0.00 ± 0.00 a	0.02 ± 0.00 a	0.48 ± 0.02 c	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
‘Mašanka’	0.10 ± 0.00 b	0.39 ± 0.01 e	0.41 ± 0.01 c	1.13 ± 0.01 e	0.74 ± 0.02 s	0.72 ± 0.01 e	0.09 ± 0.01 b	0.04 ± 0.00 b	0.03 ± 0.00 b
‘Austrougarka’	0.05 ± 0.00 a	0.24 ± 0.01 c	0.00 ± 0.00 a	1.24 ± 0.01 f	0.54 ± 0.01 f	0.00 ± 0.00 a	0.04 ± 0.00 b	0.00 ± 0.00 a	0.00 ± 0.00 a
‘Jonagold’	0.60 ± 0.01 d	0.61 ± 0.00 f	0.10 ± 0.00 b	0.14 ± 0.00 a	0.16 ± 0.00 b	0.14 ± 0.01 c	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
‘Golden Delicious’	0.14 ± 0.01 c	0.69 ± 0.02 s	0.10 ± 0.01 b	0.19 ± 0.00 a,b	0.26 ± 0.00 c	0.08 ± 0.01 b	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
‘Fuji’	0.02 ± 0.00 a	0.14 ± 0.01 b	0.10 ± 0.00 b	0.20 ± 0.02 b	0.20 ± 0.02 b,c	0.12 ± 0.01 c	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
‘Granny Smith’	0.15 ± 0.01 c	0.31 ± 0.01 d	0.12 ± 0.00 b	0.52 ± 0.03 d	0.33 ± 0.03 e	0.23 ± 0.03 d	0.06 ± 0.01 b	0.05 ± 0.01 b	0.05 ± 0.00 b

Mean $\pm$ SD based on three juice samples ($n = 3$). Within the same column, means followed by different letters are significantly different at $p \leq 0.05$.

Table 6 presents the concentration of non-flavonoid compounds ($\mu\text{g/mL}$) in juices obtained from traditional and conventional apple cultivars. The data revealed significant differences ($p \leq 0.05$) in the levels of trans-ferulic and *p*-coumaric acid among the different cultivars (Table S2). Additionally, traditional apple cultivars are characterized by higher levels of non-flavonoids, especially chlorogenic acid [45]. These cultivars exhibited higher concentrations of chlorogenic acid after harvest and during cold storage, ranging from 1.34 to 486.58 $\mu\text{g/mL}$, compared to conventional cultivars, which had significantly lower levels ranging from 22.03 to 140.75 $\mu\text{g/mL}$. As one of the major phenolic compounds in apples, chlorogenic acid plays an important role in the antioxidant capacity of apple juice. For trans-ferulic acid, the cultivar with the highest content after harvest was 'Ivanlija' ($0.66 \pm 0.06 \mu\text{g/mL}$), while the lowest levels were found in 'Jonagold' and 'Fuji' ($0.03 \pm 0.01 \mu\text{g/mL}$). For *p*-coumaric acid, the highest content was observed in 'Austrougarka' ($0.22 \pm 0.00 \mu\text{g/mL}$). Similarly, the highest caffeic acid content was found in 'Mašanka' ($0.47 \pm 0.04 \mu\text{g/mL}$), with the lowest in 'Jonagold' ($0.17 \pm 0.00 \mu\text{g/mL}$). During storage, the concentration of trans-ferulic, chlorogenic, and caffeic acids generally decreased, which aligns with our previous study [9]. The most significant reduction was seen with trans-ferulic acid in the cultivar 'Ivanlija' and chlorogenic and caffeic acids in 'Austrougarka'. On the other hand, during storage, *p*-coumaric acid levels increased, with the greatest rise observed in 'Mašanka'. This increase can be associated with the hydrolysis of bound phenolic forms or selective stability of *p*-coumaric acid under cold storage conditions. Overall, non-flavonoid contents were higher in juices obtained from traditional apple cultivars than those from conventional ones. These findings underscore the importance of conserving and promoting traditional apple cultivars for juice production.

Table 6. The concentration of non-flavonoids ($\mu\text{g/mL}$) in juices derived from traditional and conventional apple cultivars throughout cold storage.

Cultivars	<i>trans</i> -Ferulic Acid ($\mu\text{g/mL}$)			Chlorogenic Acid ($\mu\text{g/mL}$)			Caffeic Acid ($\mu\text{g/mL}$)			<i>p</i> -Coumaric Acid ($\mu\text{g/mL}$)		
	0	3	6	0	3	6	0	3	6	0	3	6
Storage time												
'Ilzer Rose-napfel'	0.13 ± 0.01^c	0.02 ± 0.00^a	0.04 ± 0.02^a	82.90 ± 1.19^d	7.47 ± 0.26^a	22.94 ± 0.61^b	0.53 ± 0.05^e	0.22 ± 0.01^b	0.33 ± 0.01^d	0.20 ± 0.00^c	0.03 ± 0.00^a	0.06 ± 0.01^a
'Ivanlija'	0.66 ± 0.06^d	0.05 ± 0.00^a	0.03 ± 0.00^a	58.61 ± 4.83^c	4.99 ± 0.49^a	1.34 ± 0.04^a	0.19 ± 0.00^{ab}	0.13 ± 0.03^a	$0.16 \pm 0.00^{b,c}$	0.09 ± 0.00^b	0.05 ± 0.02^{ab}	0.02 ± 0.00^a
'Mašanka'	0.11 ± 0.01^c	0.66 ± 0.07^c	0.31 ± 0.04^e	486.58 ± 5.48^f	388.17 ± 11.28^g	419.84 ± 4.28^f	0.47 ± 0.04^d	0.64 ± 0.03^e	2.64 ± 0.06^e	0.09 ± 0.01^b	0.40 ± 0.10^c	0.52 ± 0.01^d
'Austrougarka'	$0.08 \pm 0.00^{b,c}$	0.12 ± 0.01^b	0.04 ± 0.01^a	244.54 ± 1.17^e	186.58 ± 2.39^f	7.28 ± 0.22^a	1.35 ± 0.23^f	0.68 ± 0.01^e	0.04 ± 0.00^a	0.22 ± 0.00^c	0.11 ± 0.00^b	0.04 ± 0.01^a
'Jonagold'	0.03 ± 0.01^a	0.02 ± 0.00^a	0.07 ± 0.01^{ab}	31.67 ± 0.44^b	66.27 ± 1.12^c	40.32 ± 1.71^d	0.17 ± 0.00^a	$0.27 \pm 0.00^{b,c}$	0.14 ± 0.00^b	$0.05 \pm 0.00^{a,b}$	0.02 ± 0.00^a	0.05 ± 0.00^a
'Golden Delicious'	0.04 ± 0.02^a	0.03 ± 0.00^a	0.03 ± 0.00^a	52.64 ± 0.33^c	105.27 ± 2.47^d	44.46 ± 0.25^d	0.30 ± 0.00^c	0.30 ± 0.00^c	0.22 ± 0.00^c	0.01 ± 0.00^a	0.04 ± 0.00^a	0.02 ± 0.00^a
'Fuji'	0.03 ± 0.01^a	0.08 ± 0.01^{ab}	0.15 ± 0.02^d	78.92 ± 1.92^d	140.75 ± 15.55^e	70.86 ± 0.28^e	0.27 ± 0.00^c	0.44 ± 0.10^d	0.38 ± 0.00^d	0.02 ± 0.00^a	0.03 ± 0.01^a	0.11 ± 0.01^b
'Granny Smith'	0.05 ± 0.01^{ab}	0.05 ± 0.00^a	0.09 ± 0.01^b	22.03 ± 0.06^a	41.97 ± 1.56^b	39.54 ± 0.21^c	$0.23 \pm 0.02^{b,c}$	0.08 ± 0.02^a	$0.16 \pm 0.00^{b,c}$	0.07 ± 0.01^b	0.09 ± 0.00^b	0.32 ± 0.01^c

Mean $\pm$ SD based on three juice samples ($n = 3$). Within the same column, means followed by different letters are significantly different at $p \leq 0.05$.

Table 7 presents the concentrations of flavanols and dihydrochalcones in juices obtained from traditional and conventional apple cultivars. Among the analyzed cultivars, the highest concentrations of catechin, epicatechin, epigallocatechin, and phloridzin were observed in 'Mašanka', whereas juices from conventional cultivars generally exhibited the lowest levels. For instance, the lowest contents for catechin and epigallocatechin were detected in 'Golden Delicious' ($0.60 \pm 0.01 \mu\text{g/mL}$), the lowest epicatechin in 'Fuji' ($0.72 \pm 0.02 \mu\text{g/mL}$), and the lowest phloridzin in 'Jonagold' ($0.68 \pm 0.01 \mu\text{g/mL}$). Changes in flavanol and dihydrochalcone contents during storage were not uniform but depended on both compound type and cultivar. While most compounds decreased over

the six-month cold storage period in the majority of cultivars, catechin content in ‘Mašanka’ increased significantly, reaching $57.13 \pm 7.43 \mu\text{g/mL}$, whereas the lowest catechin content at this time point was observed in ‘Ivanlija’. Similar cultivar-dependent trends have been reported previously by Łysiak et al. [38]. The highest concentrations of epicatechin and epigallocatechin were detected in ‘Granny Smith’ ($42.91 \pm 3.41 \mu\text{g/mL}$) and ‘Mašanka’ ($88.45 \pm 3.29 \mu\text{g/mL}$). In contrast, the lowest concentration of epigallocatechin was found in ‘Jonagold’, ‘Fuji’, and ‘Golden Delicious’. The range of epigallocatechin in juices from traditional cultivars differed significantly from that observed in conventional ones ($p \leq 0.05$). Phloridzin content was highest in ‘Ilzer Rosenapfel’ ($7.89 \pm 0.15 \mu\text{g/mL}$), while the lowest was found in ‘Ivanlija’ ($0.53 \pm 0.05 \mu\text{g/mL}$). These results are consistent with the content of flavanols and dihydrochalcones reported in our previous study [5]. Phloridzin exhibits strong antioxidant, anti-inflammatory, and antifungal properties, contributing to the low susceptibility of apples to a major disease and reducing the need for chemical pesticides, thereby supporting more sustainable cultivation [11,46]. Afroz et al. reported that catechin has antibacterial, antioxidant, and antiviral activity [47]. Moreover, epicatechin and catechin were isolated as antifungal compounds [36]. In general, traditional apple cultivars are richer in flavanols and dihydrochalcones than conventional ones. These findings advance a more comprehensive understanding of the diverse biological activities of polyphenolic compounds in apple and their potential implications for human health.

Table 7. The concentration of flavanols and dihydrochalcones ($\mu\text{g/mL}$) in juices derived from traditional and conventional apple cultivars throughout cold storage.

Cultivars	Catechin ($\mu\text{g/mL}$)			Epicatechin ($\mu\text{g/mL}$)			Epigallocatechin ($\mu\text{g/mL}$)			Phloridzin ($\mu\text{g/mL}$)		
	0	3	6	0	3	6	0	3	6	0	3	6
Storage time												
‘Ilzer Rosenapfel’	1.50 ± 0.03^b	1.87 ± 0.04^{bc}	0.97 ± 0.26^d	5.74 ± 0.31^f	2.14 ± 0.02^d	1.98 ± 0.04^c	20.46 ± 0.48^c	6.11 ± 0.20^{ab}	5.50 ± 0.64^c	12.69 ± 0.21^d	6.28 ± 0.13^e	7.89 ± 0.15^e
‘Ivanlija’	1.53 ± 0.07^b	0.24 ± 0.22^a	0.00 ± 0.00^a	3.64 ± 0.09^d	1.72 ± 0.11^c	0.65 ± 0.02^a	1.10 ± 0.23^b	2.98 ± 0.17^a	0.42 ± 0.74^b	1.18 ± 0.13^b	0.82 ± 0.02^a	0.53 ± 0.05^a
‘Mašanka’	8.76 ± 0.54^e	10.19 ± 1.72^e	57.13 ± 7.43^g	20.22 ± 0.20^h	5.25 ± 0.49^g	4.59 ± 0.07^e	88.45 ± 3.29^e	63.45 ± 18.47^h	45.45 ± 0.13^d	13.48 ± 0.19^d	7.05 ± 0.10^f	7.69 ± 0.23^e
‘Austrougarka’	8.69 ± 0.49^e	11.28 ± 0.13^f	0.38 ± 0.01^b	16.65 ± 1.01^g	4.94 ± 0.03^f	0.30 ± 0.03^a	75.55 ± 6.19^d	27.10 ± 0.83^f	1.10 ± 0.06^b	4.85 ± 0.15^c	2.68 ± 0.04^c	1.17 ± 0.03^a
‘Jonagold’	2.42 ± 0.04^c	0.90 ± 0.10^a	0.43 ± 0.01^c	2.12 ± 0.08^c	0.52 ± 0.02^a	0.87 ± 0.02^{ab}	0.65 ± 0.05^a	10.56 ± 2.21^{be}	0.00 ± 0.00^a	0.68 ± 0.01^a	1.83 ± 0.03^b	1.18 ± 0.03^{ab}
‘Golden Delicious’	0.60 ± 0.01^a	1.23 ± 0.02^b	1.78 ± 0.06^e	1.17 ± 0.03^b	1.00 ± 0.00^b	1.09 ± 0.00^b	0.00 ± 0.00^a	8.43 ± 0.24^b	0.00 ± 0.00^a	1.67 ± 0.02^b	3.50 ± 0.05^d	1.44 ± 0.01^c
‘Fuji’	5.20 ± 0.26^d	2.06 ± 0.22^c	1.20 ± 0.02^e	0.72 ± 0.02^a	3.61 ± 0.30^e	3.16 ± 0.03^d	7.19 ± 0.50^b	13.68 ± 2.16^e	0.00 ± 0.00^a	1.91 ± 0.01^b	9.23 ± 1.01^g	6.92 ± 0.19^d
‘Granny Smith’	2.38 ± 0.04^c	4.37 ± 0.26^d	11.66 ± 0.34^f	4.05 ± 0.20^e	7.25 ± 0.17^h	42.91 ± 3.41^f	9.12 ± 0.68^b	55.10 ± 4.14^g	58.65 ± 0.59^e	1.15 ± 0.01^b	1.70 ± 0.02^a	1.53 ± 0.08^c

Mean $\pm$ SD based on three juice samples ($n = 3$). Within the same column, means followed by different letters are significantly different at $p \leq 0.05$.

The obtained results demonstrated that traditional cultivar ‘Mašanka’ had the highest TPC and TFC, whereas the conventional cultivar ‘Jonagold’ showed the lowest values. These findings indicate a clear relationship between polyphenolic content and antioxidant activity, with cultivars exhibiting higher TPC and TFC also demonstrating greater antioxidant capacity. The antioxidant activity of the juices, measured using both DPPH and ABTS assay, is presented in Table 8. Statistical analysis revealed significant differences ($p \leq 0.05$) in antioxidant activity among the examined cultivars (Table S2). The traditional apple cultivar ‘Mašanka’ showed higher antioxidant activity in both assays. In contrast, the lowest antioxidant activity was observed in conventional apple cultivars, such as ‘Jonagold’, ‘Granny Smith’, and ‘Golden Delicious’. This observation aligns with earlier studies, that report that traditional apple cultivars generally possess higher levels of antioxidant activity than conventional ones [5,11]. Changes in antioxidant activity during storage are largely influenced by the degradation of polyphenolic compounds over time. Enzymatic oxidation, polymerization reactions, and interactions with cellular components reduce the

concentration of free phenolics available to neutralize free radicals. Cultivars with initially higher TPC and TFC tend to maintain relatively higher antioxidant activity throughout storage, whereas cultivars with lower initial polyphenol content exhibit more pronounced declines. This positive correlation between polyphenol content and antioxidant capacity has been well-documented in previous research [36,37,48,49].

Table 8. Antioxidant activity of juices produced from traditional and conventional apple cultivars measured by two methods, DPPH and ABTS, expressed as mmol TE/L.

Cultivars	DPPH			ABTS		
	0	3	6	0	3	6
Storage time						
‘Ilzer						
Rosenapfel’	0.16 ± 0.01 ^b	0.10 ± 0.00 ^a	0.05 ± 0.00 ^a	0.60 ± 0.02 ^e	0.06 ± 0.01 ^a	0.09 ± 0.03 ^{a,b}
‘Ivanlija’	0.08 ± 0.00 ^a	0.10 ± 0.01 ^a	0.03 ± 0.00 ^a	0.37 ± 0.02 ^c	0.09 ± 0.01 ^a	0.02 ± 0.01 ^a
‘Mašanka’	0.27 ± 0.00 ^c	0.20 ± 0.01 ^d	0.17 ± 0.00 ^d	0.94 ± 0.01 ^f	0.34 ± 0.05 ^e	0.29 ± 0.00 ^d
‘Austrougarka’	0.25 ± 0.00 ^c	0.16 ± 0.00 ^c	0.04 ± 0.00 ^{a,b}	0.94 ± 0.05 ^f	0.33 ± 0.02 ^e	0.02 ± 0.01 ^a
‘Jonagold’	0.08 ± 0.00 ^a	0.12 ± 0.01 ^a	0.04 ± 0.00 ^{a,b}	0.14 ± 0.00 ^a	0.19 ± 0.01 ^b	0.11 ± 0.01 ^b
‘Golden						
Delicious’	0.10 ± 0.00 ^a	0.13 ± 0.01 ^{a,b}	0.05 ± 0.02 ^a	0.23 ± 0.02 ^b	0.25 ± 0.05 ^{c,d}	0.14 ± 0.02 ^b
‘Fuji’	0.14 ± 0.00 ^b	0.14 ± 0.01 ^b	0.07 ± 0.01 ^b	0.38 ± 0.08 ^c	0.35 ± 0.04 ^e	0.29 ± 0.04 ^d
‘Granny						
Smith’	0.07 ± 0.01 ^a	0.14 ± 0.02 ^b	0.11 ± 0.00 ^c	0.44 ± 0.02 ^d	0.30 ± 0.01 ^{d,e}	0.21 ± 0.03 ^c

Mean ± SD based on three juice samples ($n = 3$). Within the same column, means followed by different letters are significantly different at $p \leq 0.05$.

4. Conclusions

With increasing consumer awareness of the health benefits associated with dietary antioxidants and bioactive compounds, there is a growing interest in foods naturally rich with these constituents. This study provides a comprehensive comparison of apple juices produced from traditional and conventional cultivars, examining differences in pH, total acidity, sugar content (glucose, fructose, sucrose), TSS, antioxidant activity, total polyphenol and flavonoid content, and individual polyphenol profile. Juices from traditional apple cultivars generally demonstrated higher levels of quercetin, myricetin, procyanidins, phloridzin, catechin, as well as total polyphenol and flavonoid content, both immediately after harvest and throughout six months of cold storage. Correspondingly, antioxidant activity was higher in juices from traditional cultivars compared to those from conventional ones. A notable example is the traditional apple cultivar ‘Mašanka’, which not only contained higher levels of total polyphenols and flavonoids, but also demonstrated greater antioxidant activity. Despite the losses of bioactive compounds during storage, traditional apple cultivars maintained relatively higher levels of bioactive compounds and antioxidant activity throughout storage, highlighting their superior nutritional potential. These findings emphasize the importance of conserving and promoting traditional apple cultivars for juice production, as their enhanced bioactive profile and sustained antioxidant properties may provide greater health benefits to consumers.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/applbiosci5020033/s1>, Table S1: Descriptive statistical analysis; Table S2: Statistical analysis according to apple variety.

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