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Influence of 1-Methylcyclopropene on the Antioxidants of 'Red Cap' Apples during Transportation and Shelf Life

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Abstract: The dietary properties of apples make them, along with the other fruits and vegetables, the basis of many slimming or pro-health diets. Availability of apples throughout the year is ensured by various storage technologies, including the use of ripening inhibitors. This experiment focused on the assessment of the effect of various variants of inhibition of apple ripening processes, i.e., 1-methylcyclopropene (1-MCP), ultra-low-oxygen storage (ULO) or modified atmosphere (MAP), in order to provide the consumer with apples with comparable high nutritional values. An important aim of the experiment was to determine the effect of the above-mentioned factors on changes in the content of polyphenols and antioxidant capacity in conditions of apple distribution at high temperatures, i.e., above 25 °C. The experiment consisted of several stages of fruit distribution: (I)—treatment of 1-MCP apples immediately after harvest, (II)—storage in ULO, (III)—simulated long-distance transport under normal atmosphere cold storage (NA) and Modified Atmosphere Packaging (MAP), (IV)—simulated rotation (15 days) under high-temperature conditions of 25 °C. Evaluation gave the basic characteristics of the fruits that characterize their health-promoting properties, i.e., total polyphenols (TPC), phenolic acids and flavonols, and antioxidant activity (AA). All indicators were assessed separately for apple peel and flesh. The experiment showed that the content of antioxidants in apple peel is from 230 to 370% higher than in the flesh, depending on the group of ingredients assessed. The peel of fruit treated with 1-MCP was distinguished by a higher content of phenolic acids and flavonols than the untreated fruit, especially after 20 weeks of stored in ULO. The effect of 1-MCP on AA in the peel of the fruit was moderate; however, apples untreated with 1-MCP were more likely to lose AA, especially when transported under normal cold storage conditions. The content of assessed compounds in the apple flesh was more stable than in the peel. The content of TPC and phenolic acids in apple flesh either decreased or remained almost unchanged after 15 days of shelf life. An increase in AA was observed in fruit flesh not stored in ULO, especially in apples treated with 1-MCP. After 10 and 20 weeks of storage in ULO, AA was not determined by experimental factors. The use of 1-MCP and the transport of apples in MAP can reduce the loss of phenolics after long-distance transport and distribution.

Keywords: Red Cap; 1-MCP; ULO; MAP; shelf-life; total polyphenol content; antioxidant activity

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1. Introduction

Apples are the basis of many slimming or pro-health diets [1,2]. Their dietary properties result from their composition, namely because they are rich in, e.g., fiber. A single average-size apple may contain up to approx. 4 g of fiber, which is about 17% of our daily needs for that component. There are also many vitamins and minerals in apples, and they are a good source of antioxidants, including vitamin C. Ascorbic acid is an important antioxidant that has numerous functions in the human body [3]. Apples are rich in

secondary metabolites that are beneficial for our health, such as quercetin or phloridzin, which have anti-inflammatory, antiviral and anticancer effects [4–6]. Compounds from the flavonol group show antidepressant effects [7]. Free radicals left over from cellular respiration or active oxygen species can damage DNA, thus increasing the risk of cancer and degenerative changes. Compounds with antioxidant activity, such as ascorbic acid or anthocyanins, prevent this process. There are numerous antioxidant compounds in fruits, including a large group of polyphenols in the form of simple phenols, benzoic acids, phenyl propanoids and flavonoids [8]. As indicated by the literature data, apples are dominated by two groups of polyphenols—flavonoids and phenolic acids [9,10]. The type and content of polyphenols vary depending on the part of the fruit. The rind contains significant amounts of flavonols, including quercetin glycosides [9]. The flesh is rich in phenolic acids, and the seeds are abundant in dihydrochalkones [11].

Many factors influence the content of polyphenols in apples. Their content is largely a function of variety [12,13], the degree of fruit maturity [14] and part of the fruit [6,15]. Further important factors are the methods and time of storage or treatment of post-harvest fruit. These factors influence the content of polyphenolic compounds in fruits [14,16,17]. Due to the evidence that polyphenol content is largely cultivar-dependent, different results have been described in the literature dealing with apple fruit postharvest storage. Carbone et al. [16] showed that total phenol content was dramatically reduced after cold storage (1 °C for three months) in flesh (50%) and peel (20%) of "Braeburn", but not in the "Golden Delicious" and "Fuji". Moreover, Kolniak-Ostek et al. [18] showed that in apples stored for 6 months, the total concentration of polyphenols decreased to 27%, depending on the variety. However, Napolitano et al. [19] observed an increase in catechin and phloridzin in the flesh, as well as in antioxidant activity, after cold storage of Italian cultivar "Annurca". Generally, low oxygen conditions (ultra-low-oxigen (ULO), controlled-atmosphere (CA), dynamic-controlled-atmosphere (DCA)) reduce metabolism in apples. Stanger et al. [20] showed increase total phenolic compounds content in the flesh after CA and ULO storage. However, Fawbush et al. [21] found that total phenolic and flavonoid were relatively stable during storage in air and CA. In these studies, there were no correlations found between total phenolics and antioxidant activity. According to Putnik et al. [22], phenolic compounds were stable but antioxidant capacity decreased during storage of fresh-cut apples. During a long-term cold storage period, antioxidant activity decreased in both peel and flesh tissues [14]. During shelf life (20 °C) for two weeks in the studies by Matthes and Schmitz-Eiberger [23], it was shown that the content of phenols did not change in the majority of tested cultivars. Only an increase in the content of these compounds was observed in the "Wellant", while a significant decrease in the phenol content was found in the "Topaz" compared to the one measured during harvest.

A commonly used ethylene inhibitor in apples is 1-methylcyclopropene (1-MCP). According to Yurong et al. [14], the effect of 1-MCP on the content of phenolic compounds was different depending on the tested apple cultivars. MacLean et al. [17] found that in the peel of "Delicious" apples, concentration of chlorogenic acid was lower after treatment with 1-MCP than in untreated fruit. Hoang et al. [24] and Kolniak-Ostek et al. [18] claim that the 1-MCP treatment delays the loss of phenolic compounds in apples of "Cripps Pink" or "Idared" during storage but does not inhibit this process in the fruit of 'Šampion'. According to MacLean et al. [17], the use of 1-MCP prevents the degradation of anthocyanins but does not affect the content of flavonols and flavan-3-ols. The loss of overall antioxidant capacity begins when the integrity of the cell is broken due to mechanical damage or the natural aging process. The use of inhibitors, i.e., aminoethoxyvinylglycine (AVG) or 1-methylcyclopropene (1-MCP) effectively blocks aging or oxidation processes [25,26]. Commencement of catalysis of transformations and degradation of phenolic compounds by enzymes such as esterases, glycosidases and decarboxylases causes a significant loss of health-promoting properties by fruit.

Such a large variability of factors that affect content of phenolic compounds makes it difficult to compare data in the literature, because authors focus on the main quantitative

and qualitative changes in phenolic compounds during the storage and distribution of fresh fruit. In the present experiment, using various variants of inhibition of ripening processes, i.e., 1-MCP or low oxygen level during apple storage (ULO), the focus was on providing consumers with fruits of comparable high nutritional values as would be found after harvesting. An additional goal was to determine the effect of applied factors on changes in the content of polyphenols and antioxidant activity in conditions of apple distribution at high temperatures, i.e., above 25 °C.

2. Materials and Methods

2.1. Experimental Procedures

The studies were carried out to evaluate the health-promoting properties, including the analysis of the antioxidant activity, of "Red Cap" apples during simulated distribution. The fruit originated from the orchard of Warsaw University of Life Sciences located in Warsaw-Wilanów (52°14′ N 21°71′ E).

The harvest time was based on the Streif index assessment. Apples were stored in the experimental storage chambers of the Institute of Horticultural Sciences of Warsaw University of Life Sciences. Immediately after harvesting, the bottoms of the 2 halves were split to form two combinations (I)—apples untreated with 1-MCP, (II)—apples treated with 1-MCP (SmartFresh ProTabs ™, AgroFresh Solutions Inc., Philadelphia, PA, USA) at a concentration of 0.65 µL/L.

In the first stage of the experiment, apples from both combinations (with 1-MCP and without 1-MCP) were stored in Ultra-Low Oxygen (ULO) conditions with gas concentrations of 1.2% CO_2 and 1.2% O_2 (temperature 1 °C and relative humidity approx. 95%). The storage period of apples in ULO was 0 (apples designed directly to the simulated transport—stage 2), 10 and 20 weeks (two groups of postharvest treatment per three periods of storage).

The second stage of the experiment was simulated fruit transport. At this stage, the fruit was packed into selected gas permeability bags, intended for storing apples (Xtend®, by StePac L.A. Ltd., Tefen, Israel), to provide Modified Atmosphere Packaging (MAP) or into cardboard boxes with no dedicated packaging. The packages with apples were placed in an ordinary cold storage (temperature 1 °C), thus obtaining the next two combinations, i.e., transport in MAP and transport in a standard cold storage (NA). In the second stage, 4 combinations of the experiment were obtained (two groups of postharvest treatment per two technologies of simulated long-distance transport per two periods of simulated long-distance transport):

Apples treated 1-MCP transported in NA
Apples non-treated 1-MCP transported in NA
Apples treated 1-MCP transported in MAP
Apples non-treated 1-MCP transported in MAP.

The period of simulated trading was 6 and 8 weeks.

The third stage of the experiment was shelf life (SL). After simulated long-distance transport, apples were subjected to simulated distribution, which was conducted at a temperature of 25 °C, which was applied for 0 and 15 days. The evaluation of secondary metabolites content and antioxidant capacity of the fruit was carried out immediately before the simulated distribution (0 days) and after 15 days of shelf life.

Each studied group consisted of three batches, with 10 apples each, and the procedures applied in the experimental groups are presented in Figure 1.

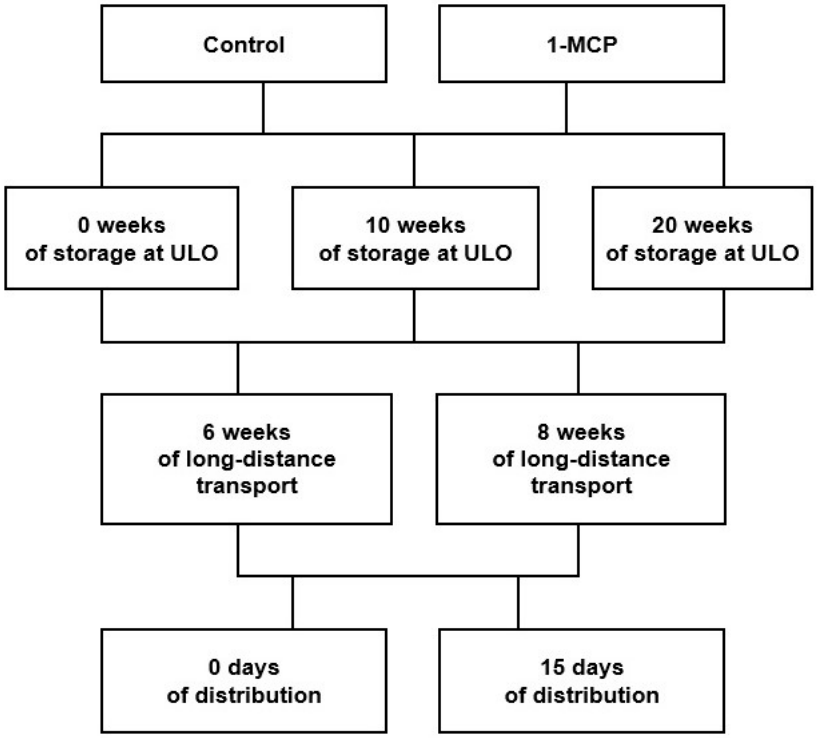


Figure 1. The studied groups of "Red Cap" apples. Note: 1-MCP—1-methylcyclopropene; ULO—ultra-low-oxigen

2.2. Analytical Methods

The apples were studied after simulated distribution to assess total polyphenols content (TPC), phenolic acids, flavonols and antioxidant activity (AA) in 24 groups of postharvest treatment/ULO storage/long-distance transport/distribution.

All reagents were of analytical purity gradients or HPLC grade purchased by Sigma-Aldrich (Poznań, Poland) or Merck (Warsaw, Poland).

The total polyphenol content (TPC) was determined by the spectrophotometric method [27] using the Folin–Ciocalteu reagent. The absorbance of the solution was measured in a spectrophotometer Marcel 330S PRO (Marcel, Poland) at the wavelength λ = 700 nm. Presented results were recalculated into gallic acid (mg·100 g^{-1} F.W.).

Phenolic compounds were separated using the HPLC technique described in our previous studies [28]. Analysis of the separation and contents of phenolic compounds was performed using a Perkin-Elmer 200 series HPLC kit with a Diode Array Detector (DAD). Separation was carried out using a LiChroCART 125-3 (Merck KGaA, Darmstadt, Germany) column with a 1 mL/min flow rate. The column temperature was 22 °C. The mobile phase consisted of a water (A): 20% formic acid (B): acetonitrile (C) mixture at various concentration gradients. Phenolic compounds were detected at 280, 300, 320 and 360 nm wavelengths by comparing retention times on achieved chromatograms with standard ones. Contents of particular compounds (total of two groups: phenolic acids and flavonols) was given in (mg·100 g^{-1} F.W.).

The antioxidant activity was determined according to the method by Saint Criq de Gaulejac et al. [29] with the help of the synthetic radical DPPH (1,1-diphenyl-2-picrylhydrazine, Sigma-Aldrich, Poznań, Poland). The antioxidant activity was calculated on the basis of absorbance measurements for the proper sample (fruit extract + DPPH+) performed after 20 min at λ = 517 nm in relation to the control sample (H2O + DPPH+). Results were expressed in mg of ascorbic acid equivalent (AAE) per g of F.W. (mg AAE·100 g^{-1} F.W.).

The internal ethylene content (IEC) (µL/L) was assessed according to a widely applied methodology, similarly as in the previous studies [30]. It was measured in the core space of apples while using a 1 mL syringe to collect samples of air. For each apple, 1 mL of air

was injected and assessed while using the gas chromatography (HP 5890, Hewlett Packard, Palo Alto, CA, USA) for ethylene analysis.

The starch index (SI) was assessed according to a widely applied methodology, similarly as in our own previous studies [30]. It was based on the reaction with the Lugol's solution and assessed visually in comparison with the 10-points scale standards.

The fruit samples, separately taken from peel and for flesh, were immediately frozen in liquid nitrogen after collection and stored (−80 °C). The peel of apples was taken from opposite sides of the fruit, vertically (from top to bottom). All peel fragments of 10 fruits from the replicate (mixed test) were ground in an IKA A11 grinder (IKA Werke, Staufen, Denmark) in liquid nitrogen. Fruit flesh was sampled similarly, cutting 2 opposite segments from 10 apples in replicate.

2.3. Statistical Analysis

The results were analyzed statistically in program Statistica 12.5 (StatSoft Polska, Krakow, Poland) using the three-way analysis of variance. Tukey test was used for evaluation of the significance of differences between the means, accepting the significance level as 5%. Principal Component Analysis (PCA) was performed using XLSTAT statistical software (Addinsoft, France, Paris).

3. Results

Characteristics of antioxidant properties and physiological maturity parameters directly after harvest of 'Red Cap' apple are presented in Table 1.

Table 1. Characteristics of "Red Cap" apples assessed directly after harvest.

Characteristics	Part of fruit	Mean ± SD
Internal ethylene content (μL/L)		1.86 ± 5.1
Starch index (−)		7.6 ± 1.0
TPC (mg·100 g^{-1} F.W.)	peel flesh	390 ± 31.6 193 ± 14.2
Phenolic acids (mg·100 g^{-1} F.W.)	peel flesh	43.6 ± 2.8 12.4 ± 0.9
Flavonols (mg·100 g^{-1} F.W.)	peel flesh	316 ± 27.4 164 ± 13.8
AA (mg AAE·100 g^{-1} F.W.)	peel flesh	73.6 ± 6.6 24.1 ± 1.9

Total polyphenol content was determined by the use of 1-MCP after fruit harvest. The peel of 1-MCP-treated fruit was distinguished by a higher TPC of the untreated fruit (Table 2). This relationship was observed especially in apples that were not stored in ULO. Along with the extension of the ULO storage period, this relationship disappeared. The positive effect of 1-MCP on the content of TPC was observed even after 15 days of SL but only in apples not stored or stored in ULO for 10 weeks. The use of 1-MCP did not significantly affect the TPC in apple flesh. Some differences in the TPC of apple flesh treated versus untreated with 1-MCP do not allow us to specify an explicit argument for the effects of the compound. The influence of high temperatures during shelf life on the content of TPC has not been clearly proven. Most analyzes demonstrated an increase in polyphenols in the peel of apples, but only in a few cases was it confirmed statistically. Generally, it can be concluded that the content of TPC increased in the peel of treated fruit after harvesting with 1-MCP, while in untreated fruit, this relationship was not observed. Furthermore, directions of changes in the content of polyphenols in apple flesh during the simulated rotation do not allow for a clear description of the relationships. TPC in apple flesh either decreased or remained almost unchanged after 15 days shelf life.

Table 2. The TPC (mg·100 g^{-1} F.W.) of "Red Cap" apples after 1-MCP treatment, ultra-low-oxygen (ULO) storage, simulated long-distance transport, and simulated distribution.

Period and Conditions of Long-Distance Transport		Postharvest Treatment	Period of Simulated Distribution (Days)					
			0	15	Significance	0	15	Significance
			Peel			Flesh		
Period of Storage in ULO—0 Weeks								
6 weeks	NA	+1-MCP	366 ± 33	405 ± 11	**	201 ± 14	190 ± 19	**
		−1-MCP	339 ± 22	352 ± 22	ns	196 ± 13	189 ± 13	*
		Significance	**	**		ns	ns	
	MAP [1]	+1-MCP	458 ± 58	424 ± 31	ns	189 ± 15	199 ± 20	**
		−1-MCP	391 ± 36	387 ± 23	ns	187 ± 12	195 ± 13	*
		Significance	**	**		ns	ns	
8 weeks	NA	+1-MCP	393 ± 20	425 ± 34	**	197 ± 13	193 ± 14	ns
		−1-MCP	394 ± 11	396 ± 20	ns	195 ± 13	186 ± 15	**
		Significance	ns	*		ns	*	
	MAP	+1-MCP	402 ± 33	442 ± 24	**	196 ± 12	188 ± 12	*
		-1-MCP	388 ± 21	407 ± 27	ns	194 ± 12	186 ± 15	*
		Significance	ns	*		ns	ns	
Period of Storage in ULO—10 Weeks								
6 weeks	NA	+1-MCP	409 ± 12	441 ± 38	**	162 ± 11	152 ± 14	**
		−1-MCP	357 ± 30	394 ± 35	**	157 ± 13	152 ± 13	ns
		Significance	**	**		ns	ns	
	MAP	+1-MCP	442 ± 18	470 ± 18	**	151 ± 11	153 ± 14	ns
		−1-MCP	420 ± 36	411 ± 24	ns	156 ± 15	156 ± 13	ns
		Significance	ns	**		ns	ns	
8 weeks	NA	+1-MCP	441 ± 30	458 ± 38	ns	157 ± 16	158 ± 12	ns
		−1-MCP	432 ± 38	427 ± 19	ns	155 ± 14	163 ± 21	*
		Significance	ns	*		ns	ns	
	MAP	+1-MCP	430 ± 34	483 ± 11	**	154 ± 11	156 ± 19	ns
		−1-MCP	418 ± 18	438 ± 28	ns	157 ± 21	146 ± 17	**
		Significance	ns	**		ns	**	
Period of Storage in ULO—20 Weeks								
6 weeks	NA	+1-MCP	338 ± 36	359 ± 38	ns	159 ± 20	157 ± 20	ns
		−1-MCP	314 ± 21	342 ± 27	**	161 ± 17	154 ± 16	*
		Significance	ns	**		ns	ns	
	MAP	+1-MCP	274 ± 33	333 ± 37	**	155 ± 15	141 ± 14	**
		−1-MCP	295 ± 18	309 ± 25	ns	158 ± 13	154 ± 16	ns
		Significance	ns	Ns		ns	**	
8 weeks	NA	+1-MCP	352 ± 34	380 ± 31	ns	157 ± 14	160 ± 14	ns
		−1-MCP	377 ± 25	364 ± 18	ns	151 ± 14	151 ± 14	ns
		Significance	ns	Ns		*	**	
	MAP	+1-MCP	363 ± 26	389 ± 11	ns	158 ± 20	157 ± 17	ns
		−1-MCP	340 ± 18	357 ± 23	ns	153 ± 11	159 ± 15	ns
		Significance	ns	*		ns	ns	

[1] MAP—modified atmosphere; NA—normal atmosphere cold storage; 1-MCP—1-methylcyclopropene; ±—standard deviation; statistically significant difference (Tukey's range test): *—for 5%. **—for 1%. for comparing the averages: impact of 1-MCP (line) and simulated distribution of period (column); ns—lack of statistical significance.

In general, it can be concluded that the apples treated with 1-MCP had a higher content of phenolic acids in the peel than the untreated apples. In fruits that were transported immediately after harvest (no storage at ULO) the effect of 1-MCP treatment was mainly marked after 15 days of shelf life (Table 3). After 10 weeks of storage at ULO it was noticed that the phenolic acid content in the peel of the 1-MCP-treated apples was significantly higher especially in NA. Again after 15 days of SL the beneficial effect of 1-MCP was observed. Long-term storage of apples in ULO chamber resulted in an overall decrease in

the content of phenolic acids in the skin of the fruit. In this case, the peel of fruit treated with 1-MCP was characterized by a much higher content of phenolic acids than that of the untreated fruit regardless of period and conditions of transport. The influence of 1-MCP on the content of the discussed index in apple flesh was insignificant. Only after 20 weeks of storage of apples in ULO and 8 weeks of transport in NA was a positive effect of post-harvest treatment with 1-MCP on the content of phenolic acids observed. The experiment revealed a negative effect of the period of simulated trade on the content of phenolic acids in apple peel. After 15 days of SL, a decrease in the content of phenolic acids was found, but in apples treated with 1-MCP, it was much lower. On the other hand, the content of phenolic acids in apple flesh seems to be more stable. A significant loss of compounds was found in a few cases of apples transported directly after harvest. However, in the flesh of apples stored in ULO for 10 or 20 weeks, there was no significant reduction of phenolic acids after 15 days SL.

The effect of 1-MCP on the content of flavonols was variable. The peel of apples transported directly after harvesting under CA conditions showed a higher content of flavonols if they were untreated with 1-MCP (Table 4). However, after 15 days of SL, the flavonols content in the peel was higher in apples treated with 1-MCP regardless of the conditions in which fruit was transported. Storage of apples treated with 1-MCP inhibited the loss of flavonols in their peel, which is clearly visible after 20 weeks of storage in ULO regardless of the conditions in which the fruit was transported. The disproportion in the content of flavonols in the peel of apples increased after a period of 15 days SL. After this time, the apples had a significantly higher content of compounds in the peel if they were treated with 1-MCP after harvest compared to the apples untreated with 1-MCP. The period of the simulated turnover did not unequivocally determine the loss or increase of flavonols in the peel. In general, the flavonols content in the peel of apples treated with 1-MCP increased after 15 days SL, although this was not always statistically proven, while the flavonols in the peel of apples untreated with 1-MCP decreased their concentration. The content of flavonols in the apple flesh was very stable and neither the treatment of 1-MCP fruits the conditions in which the fruits were transported nor the SL period determined their quantity.

The experiment proved that post-harvest treatment of apples with 1-MCP contributed to a lower antioxidant activity (AA) in fruit peel. This trend was clearly visible in the fruit transported immediately after harvest and in the fruit after 10 weeks of storage (in ULO) transported under the conditions of regular cold storage (Table 5). Unexpectedly, the longer storage of apples in ULO, i.e., for a period of 20 weeks, eliminated the effect of 1-MCP, and AA in the peel of 1-MCP-treated and -untreated fruit was similar. After 15 days, the SL effect of 1-MCP was no longer so clear. After this period, it was noted that the effect of 1-MCP was strong in apples not stored compared to apples stored for 20 weeks at ULO. The effect of shelf life was dependent on the treatment of 1-MCP fruit and transport conditions. In the fruit transported directly after harvest, the directions of changes in AA did not allow for defining a clear relationship. However, a trend emerged in the fruit stored, which is particularly visible in apples stored for 20 weeks at ULO. The fruits treated with 1-MCP showed little or no change in AA in the peel. In contrast, fruit untreated with 1-MCP more often was characterized by loss of AA in the peel especially if transported under ordinary cold storage conditions. AA in the fruit flesh was determined by the treatment with 1-MCP as well as the apple transport conditions. While after 20 weeks of storage in ULO the effect of 1-MCP in the peel was not noticeable, in the flesh, this effect was very significant. Fruits treated with 1-MCP in all transport combinations were characterized by higher AA. This relationship was proved both before and after the SL period. With the shorter shelf life of apples at ULO i.e., 0 and 10 weeks, the flesh of the fruit treated with 1-MCP was characterized by higher AA only after 15 days of shelf life. The impact of the SL period was variable. Increases in AA were noted in fruit flesh not stored at ULO, especially in apples treated with 1-MCP. Unfortunately, this trend was reversed at subsequent analysis

dates, and in the flesh of apples after 20 weeks of storage in ULO, a decrease in AA was noted although statistically proven only in a few cases.

Table 3. The phenolic acid content (mg·100 g^{-1} F.W.) of "Red Cap" apples after 1-MCP treatment, ultra-low-oxygen (ULO) storage, simulated long-distance transport, and simulated distribution.

Period and Conditions of Long-Distance Transport		Postharvest Treatment	Period of Simulated Distribution (Days)					
			0	15	Significance	0	15	Significance
			Peel			Flesh		
Period of Storage in ULO—0 Weeks								
6 weeks	NA	+1-MCP	44.1 ± 1.0	34.1 ± 1.1	**	12.6 ± 0.5	9.4 ± 0.8	*
		−1-MCP	38.5 ± 2.1	26.5 ± 3.1	**	9.6 ± 2.0	9.7 ± 0.8	ns
		Significance	**	**		*	ns	
	MAP [1]	+1-MCP	44.1 ± 2.8	40.8 ± 2.5	ns	12.1 ± 0.7	13.0 ± 0.7	ns
		−1-MCP	44.9 ± 2.0	28.5 ± 0.7	**	12.2 ± 0.4	12.8 ± 0.4	ns
		Significance	ns	**		ns	ns	
8 weeks	NA	+1-MCP	40.1 ± 3.8	34.0 ± 3.4	*	14.3 ± 0.5	13.1 ± 0.2	ns
		−1-MCP	40.7 ± 4.3	39.0 ± 3.5	ns	14.5 ± 0.4	10.2 ± 0.4	*
		Significance	ns	*		ns	*	
	MAP	+1-MCP	56.0 ± 2.4	42.4 ± 0.3	**	13.5 ± 0.6	11.0 ± 0.5	ns
		−1-MCP	54.7 ± 3.2	38.4 ± 3.1	**	13.6 ± 0.2	9.9 ±0.8	**
		Significance	ns	*		ns	ns	
Period of Storage in ULO—10 Weeks								
6 weeks	NA	+1-MCP	51.4 ± 5.4	47.8 ± 1.5	*	10.4 ± 0.6	7.9 ± 0.1	**
		−1-MCP	46.3 ± 5.2	34.2 ± 1.2	**	8.1 ± 1.4	8.2 ± 0.7	ns
		Significance	*	**		*	ns	
	MAP	+1-MCP	47.8 ± 3.3	45.2 ± 1.9	ns	9.8 ± 0.2	7.9 ± 0.1	ns
		−1-MCP	46.0 ± 3.2	30.5 ± 0.6	**	10.5 ± 0.2	9.8 ± 0.4	ns
		Significance	ns	**		ns	ns	
8 weeks	NA	+1-MCP	48.5 ± 2.3	42.9 ± 3.9	*	8.4 ± 0.9	10.0 ± 0.1	ns
		−1-MCP	43.1 ± 3.9	42.8 ± 4.4	ns	9.4 ± 0.6	11.1 ± 0.2	ns
		Significance	*	ns		ns	ns	
	MAP	+1-MCP	58.0 ± 0.6	45.9 ± 0.8	**	10.1 ± 0.4	9.5 ± 0.7	ns
		−1-MCP	59.6 ± 2.9	41.3 ± 3.3	**	10.6 ± 0.4	7.6 ± 0.2	**
		Significance	ns	*		ns	ns	
Period of Storage in ULO—20 Weeks								
6 weeks	NA	+1-MCP	42.7 ± 1.5	30.0 ± 1.9	**	11.2 ± 0.2	10.3 ± 0.2	ns
		−1-MCP	24.9 ± 0.9	19.9 ± 1.2	*	12.1 ± 0.2	9.4 ± 1.3	ns
		Significance	**	**		ns	ns	
	MAP	+1-MCP	44.0 ± 0.1	33.5 ± 2.0	**	9.1 ± 0.2	9.0 ± 0.4	ns
		−1-MCP	26.0 ± 0.2	15.1 ± 1.6	**	11.2 ± 0.4	10.5 ± 0.2	ns
		Significance	**	**		ns	ns	
8 weeks	NA	+1-MCP	31.2 ± 0.8	30.5 ± 1.2	ns	10.7 ± 0.2	10.1 ± 0.4	ns
		−1-MCP	23.0 ± 0.7	19.1 ± 1.1	*	8.1 ± 0.6	8.3 ± 0.3	ns
		Significance	**	**		*	*	
	MAP	+1-MCP	29.1 ± 0.9	27.3 ± 1.4	ns	10.4 ± 0.2	10.3 ± 0.2	ns
		−1-MCP	23.9 ± 1.5	15.8 ± 1.5	**	8.9 ± 0.4	9.7 ± 0.1	ns
		Significance	**	**		ns	ns	

[1] MAP—modified atmosphere; NA—normal atmosphere cold storage; 1-MCP—1-methylcyclopropene; ±—standard deviation; statistically significant difference (Tukey's range test): *—for 5%. **—for 1%. for comparing the averages: impact of 1-MCP (line) and simulated distribution of period (column); ns—lack of statistical significance.

Table 4. The flavonols content (mg·100 g^{-1} F.W.) of "Red Cap" apples after 1-MCP treatment, ultra-low-oxygen (ULO) storage, simulated long-distance transport, and simulated distribution.

Period and Conditions of Long-Distance Transport		Postharvest Treatment	Period of Simulated Distribution (Days)					
			0	15	Significance	0	15	Significance
			Peel			Flesh		
Period of Storage in ULO—0 Weeks								
6 weeks	NA	+1-MCP	298 ± 13	324 ± 11	ns	170 ± 14	164 ± 18	**
		−1-MCP	275 ± 19	293 ± 17	ns	169 ± 15	162 ± 12	ns
		Significance	ns	**		ns	ns	
	MAP [1]	+1-MCP	327 ± 12	345 ± 27	ns	160 ± 14	167 ± 12	ns
		−1-MCP	373 ± 15	307 ± 24	**	158 ± 11	165 ± 13	*
		Significance	**	**		ns	ns	
8 weeks	NA	+1-MCP	324 ± 15	346 ± 27	ns	164 ± 13	162 ± 14	ns
		−1-MCP	317 ± 18	321 ± 15	ns	163 ± 12	159 ± 15	ns
		Significance	ns	*		ns	ns	
	MAP	+1-MCP	310 ± 28	360 ± 23	**	165 ± 11	160 ± 11	ns
		−1-MCP	398 ± 38	331 ± 22	**	163 ± 11	159 ± 13	ns
		Significance	**	**		ns	ns	
Period of Storage in ULO—10 Weeks								
6 weeks	NA	+1-MCP	321 ± 13	426 ± 38	**	136 ± 11	131 ± 13	ns
		−1-MCP	290 ± 21	312 ± 16	ns	134 ± 14	130 ± 13	ns
		Significance	**	**		ns	ns	
	MAP	+1-MCP	354 ± 15	382 ± 15	ns	128 ± 11	131 ± 14	ns
		−1-MCP	351 ± 16	328 ± 24	ns	132 ± 15	132 ± 12	ns
		Significance	ns	**		ns	ns	
8 weeks	NA	+1-MCP	352 ± 19	374 ± 30	ns	135 ± 16	134 ± 12	ns
		−1-MCP	349 ± 13	345 ± 16	ns	132 ± 14	137 ± 12	ns
		Significance	ns	**		ns	ns	
	MAP	+1-MCP	333 ± 30	393 ± 10	**	130 ± 11	132 ± 13	ns
		−1-MCP	320 ± 19	356 ± 23	ns	132 ± 11	125 ± 11	*
		Significance	ns	**		ns	*	
Period of Storage in ULO—20 Weeks								
6 weeks	NA	+1-MCP	356 ±14	329 ± 26	ns	133 ± 12	132 ± 12	ns
		−1-MCP	323 ±16	281 ± 16	**	134 ± 18	131 ± 15	ns
		Significance	**	**		ns	ns	
	MAP	+1-MCP	325 ±14	349 ± 25	ns	132 ± 11	120 ± 13	**
		−1-MCP	235 ±17	245 ± 15	ns	132 ± 12	130 ± 19	ns
		Significance	**	**		ns	**	
8 weeks	NA	+1-MCP	320 ±29	333 ± 21	ns	132 ± 13	135 ± 14	ns
		−1-MCP	289 ±28	252 ± 16	**	129 ± 17	129 ± 14	ns
		Significance	**	ns		ns	*	
	MAP	+1-MCP	303 ±14	335 ± 19	ns	133 ± 11	133 ± 11	ns
		−1-MCP	256 ±15	217 ± 17	*	130 ± 15	135 ± 15	ns
		Significance	**	**		ns	ns	

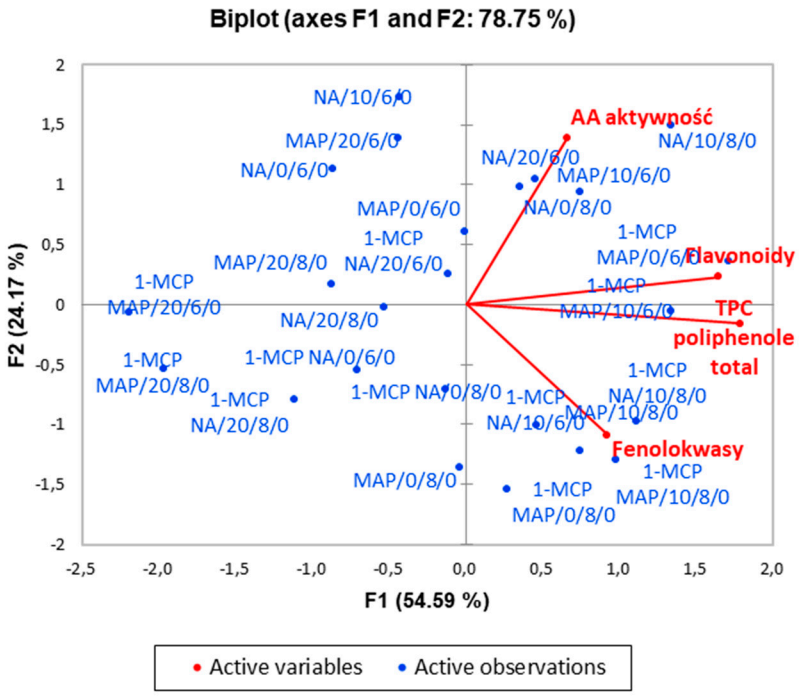
[1] MAP—modified atmosphere; NA—normal atmosphere cold storage; 1-MCP—1-methylcyclopropene; ± - standard deviation; statistically significant difference (Tukey's range test): *—for 5%. **—for 1%. for comparing the averages: impact of 1-MCP (line) and simulated distribution of period (column); ns—lack of statistical significance.

Table 5. The AA (mg·100 g^{-1} F.W.) of "Red Cap" apples after 1-MCP treatment, ultra-low-oxygen (ULO) storage, simulated long-distance transport and simulated distribution.

Period and Conditions of Long-Distance Transport		Postharvest Treatment	Period of Simulated Distribution (Days)					
			0	15	Significance	0	15	Significance
			Peel			Flesh		
Period of Storage in ULO—0 Weeks								
6 weeks	NA	+1-MCP	63.1 ± 3.4	58.8 ± 2.2	ns	30.7 ± 1.9	33.2 ± 2.8	*
		−1-MCP	91.0 ± 1.0	65.5 ± 3.0	**	28.8 ± 0.2	30.7 ± 1.1	ns
		Significance	**	*		ns	*	
	MAP [1]	+1-MCP	81.9 ± 1.7	68.8 ± 4.4	**	25.8 ± 2.2	35.9 ± 0.7	**
		−1-MCP	74.9 ± 1.2	81.6 ± 1.5	*	25.9 ± 1.1	28.2 ± 1.9	ns
		Significance	*	**		ns	**	
8 weeks	NA	+1-MCP	60.0 ± 2.7	71.2 ± 3.3	**	33.4 ± 1.2	37.7 ± 1.0	**
		−1-MCP	89.5 ± 1.7	64.3 ± 3.5	**	31.8 ± 1.3	34.0 ± 1.7	ns
		Significance	**	*		ns	*	
	MAP	+1-MCP	64.8 ± 3.7	64.5 ± 4.1	ns	32.1 ± 0.2	39.2 ± 1.9	**
		−1-MCP	66.8 ± 5.0	77.4 ± 4.7	*	30.3 ± 1.4	36.9 ± 2.9	**
		Significance	ns	**		ns	ns	
Period of Storage in ULO—10 Weeks								
6 weeks	NA	+1-MCP	68.8 ± 4.2	79.6 ± 2.4	**	28.4 ± 0.6	30.6 ± 0.9	ns
		−1-MCP	95.9 ± 5.2	70.8 ± 2.3	**	26.3 ± 0.9	28.8 ± 2.4	ns
		Significance	**	ns		ns	ns	
	MAP	+1-MCP	79.2 ± 3.3	76.0 ± 2.4	ns	24.8 ± 1.0	25.2 ± 2.8	ns
		−1-MCP	80.5 ± 4.5	86.9 ± 2.5	ns	24.8 ± 0.3	18.4 ± 2.4	**
		Significance	ns	*		ns	**	
8 weeks	NA	+1-MCP	66.5 ± 5.2	76.2 ± 2.8	*	22.7 ± 0.9	27.3 ± 0.3	**
		−1-MCP	97.9 ± 1.6	69.4 ± 5.0	**	21.1 ± 1.4	18.2 ± 1.9	**
		Significance	**	ns		ns	**	
	MAP	+1-MCP	69.3 ± 5.9	70.4 ± 2.5	ns	21.0 ± 0.5	22.4 ± 1.2	ns
		−1-MCP	71.9 ± 4.4	83.2 ± 5.7	**	20.6 ± 0.9	19.1 ± 1.1	ns
		Significance	ns	**		ns	*	
Period of Storage in ULO—20 Weeks								
6 weeks	NA	+1-MCP	71.0 ± 3.2	65.4 ± 5.5	ns	22.4 ± 0.5	18.3 ± 0.9	*
		−1-MCP	73.9 ± 4.6	66.2 ± 5.0	*	19.3 ± 0.8	15.4 ± 1.1	*
		Significance	ns	ns		*	*	
	MAP	+1-MCP	70.8 ± 1.3	70.1 ± 1.8	ns	22.2 ± 0.9	19.9 ± 0.8	ns
		−1-MCP	76.0 ± 3.2	68.3 ± 4.3	*	18.1 ± 0.2	16.6 ± 0.7	ns
		Significance	ns	ns		*	*	
8 weeks	NA	+1-MCP	57.6 ± 4.1	57.6 ± 1.3	ns	20.4 ± 1.0	18.7 ± 0.8	ns
		−1-MCP	63.8 ± 4.7	55.4 ± 5.9	*	15.7 ± 0.6	12.7 ± 0.2	*
		Significance	ns	ns		**	**	
	MAP	+1-MCP	60.4 ± 3.0	58.3 ± 2.0	ns	20.5 ± 0.5	18.2 ± 0.6	ns
		−1-MCP	65.6 ± 1.5	56.0 ± 2.9	*	15.8 ± 0.5	15.4 ± 0.4	ns
		Significance	ns	ns		**	*	

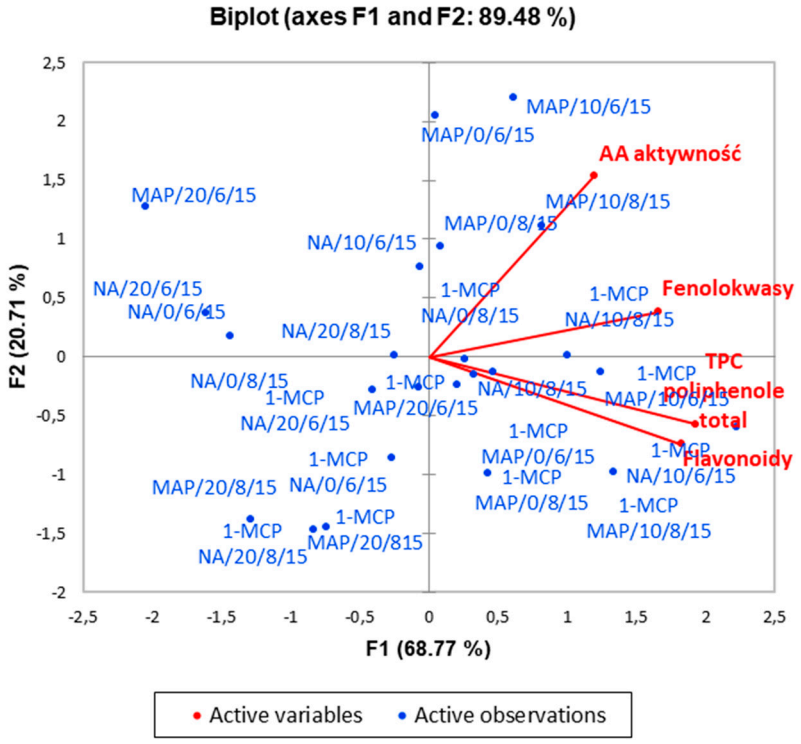
[1] MAP– modified atmosphere; NA— normal atmosphere cold storage; 1-MCP—1-methylcyclopropene; ±—standard deviation; statistically significant difference (Tukey's range test): *—for 5%. **—for 1%. for comparing the averages: impact of 1-MCP (line) and simulated distribution of period (column); ns—the lack of statistical significance.

Principal component analysis was applied to explore the differences and similarities in the contents of phenolic compounds and antioxidant capacity in apple peel and apple flesh, taking into account the experimental variables (Figure 2A,B and Figure 3A,B). The results of PCA for the examined compounds in apple peel explained 78.75% and 89.48% of the total variance for the first two principal components, taking into account 0 days and 15 days of simulated distribution (Figure 2A,B). The peel of apples treated with 1-MCP and MAP during 0 and 10 days of storage in ULO were characterized by a higher amount of phenolic acid in general. It was found that the peel of fruits without 1-MCP and transported in NA at various storage times and sample MAP/10/6/0 were associated with a higher level of antioxidant activity, while apple peel with 1-MCP and stored for 0 and 10 days in the same long-distance transport (6 weeks) were similar in the amount of TPC and flavonols. The content of phenolic compounds and level of the antioxidant activity in apple peel decreased with the extension of the storage time and transport process (Figure 2A). After 15 days of simulated distribution, the addition of 1-MCP and MAP positively influenced the contents of compounds such as phenolic acid, TPC and flavonols (Figure 2B). Samples with MAP and different storage times (0 weeks, 10 weeks) as well as transport processes (6 and 8 weeks) were more associated with the antioxidant activity. Subsequent samples contained lower amounts of TPC, flavonols and phenolic acid and showed lower AA due to storage time (20 weeks) and other variability factors in the experiment (they focused on the left side of PCA). The changes in the amount of examined compounds in the NA samples were more irregular and scattered across the PCA plot.



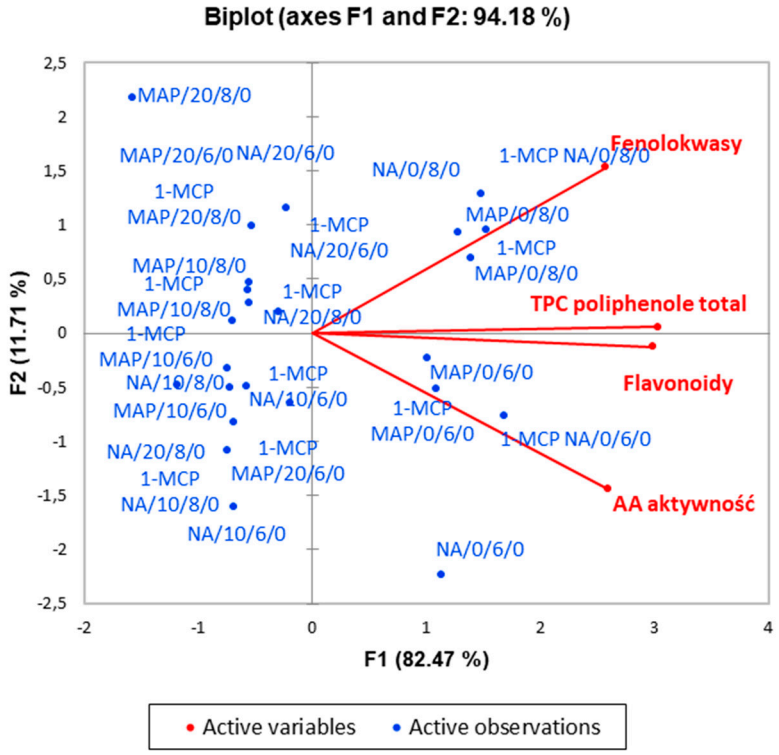
(**A**)

Figure 2. *Cont.*



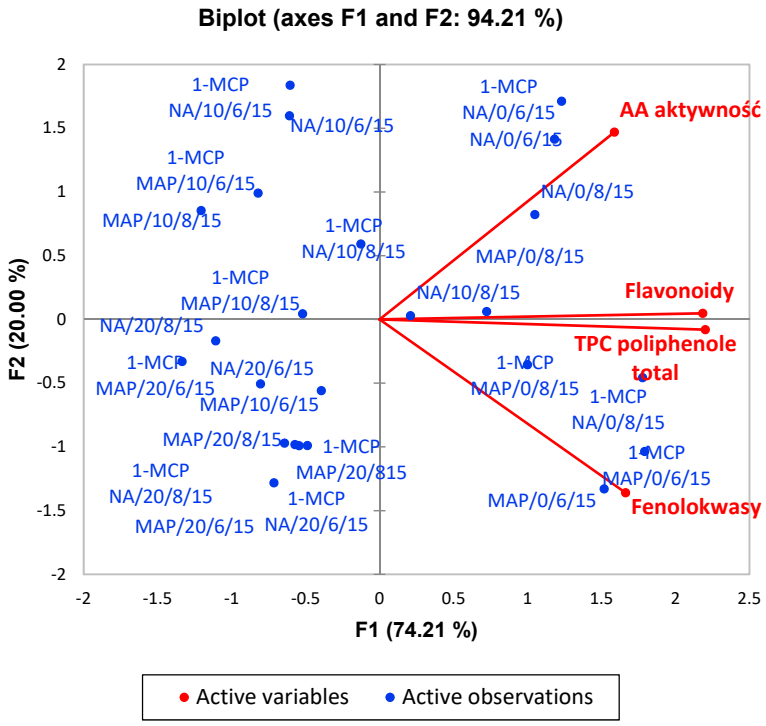
(**B**)

Figure 2. PCA biplot showing the relationship between the content of phenolic compounds and antioxidant capacity in the apple peel for (**A**) 0 days of simulated distribution and (**B**) 15 days of simulated distribution.



(**A**)

Figure 3. *Cont*.



(**B**)

Figure 3. PCA biplot showing the relationship between the content of phenolic compounds and antioxidant capacity in the apple flesh for (**A**) 0 days of simulated distribution and (**B**) 15 days of simulated distribution.

The results of the PCA for the apples' flesh revealed that the first two components describe 94.18% (in 0 days of simulated distribution) and 94.21% (in 15 of simulated distribution) of the initial variability (Figure 3A,B). Samples of the apple flesh during 0 weeks of the storage with 1-MCP and MAP as well as various transport process (6 vs. 8 weeks) contained a higher level of polyphenols and AA compared to other products (Figure 3A). A relatively large cluster of samples with a lower amount of positive compounds was observed on the opposite side of the PCA as a storage effect (10 and 20 weeks in ULO). A similar pattern of changes was noted for the apple flesh after 15 days of simulated distribution. The level of TPC, flavonols, phenolic acid and antioxidant activity was lowered due to the shelf life process.

4. Discussion

Apple fruits contain many substances that have a beneficial effect on human health [31,32]. Regular consumption of apples can contribute to mass loss, and it also helps to prevent cardiovascular disease. Both weight control and cardiovascular effects are confirmed by studies on mice that were fed with a supplement of ground apples and apple juice concentrate; these mice decreased in mass and had lower levels of LDL cholesterol, triglycerides and total cholesterol than the control group [2]. Muraki et al. [1] link apple consumption with a reduced risk of diabetes type 2. Hyson [32] and Luo et al. [6] claim that plant compounds present in apples contribute to a reduction of cancer incidence and are helpful in strengthening of the immune system as well as in respiratory diseases, such as asthma. Apples are rich in antioxidant plant compounds. Phenolic compounds are the main group of secondary metabolites responsible for the antioxidant properties of apples. Many groups of polyphenols have been identified in apples, e.g., flavonoids and phenolic acids [9,10]. Ambient temperature during transport and sale in tropical countries can significantly contribute to the loss of fruit quality. In the literature, a few reports raise issues describing

changes in the content of phenolic compounds in apples stored in a controlled atmosphere technology or treated with 1-methylcyclopropene [14,23,26].

Many people eat apples after peeling them, even though the skin is an integral part of the fruit and contains valuable compounds. Numerous studies show that apple peel is much richer in phenolic compounds than the flesh [33,34]. The polyphenol content in apples is influenced by many factors, such as the varietal factor [18,35] or the degree of maturity [14]. The results of our own experience confirm a higher content of phenolic compounds in the skin than in the flesh.

The content of phenolic acids in the tests decreased or remained unchanged during the simulated sale at high temperatures. However, during the simulated sale, an increase in the content of flavonols in the peel of apples, especially those transported for 8 weeks, was observed. On the other hand, the content of flavonols in the apple flesh was stable during the simulated turnover. Similar relationships were shown [17] in fruit subjected to simulated rotation at a temperature of about 21 °C. However, different results were obtained by Ju et al. [36] who found a decrease in the content of phenolic acids and flavonols in apples during the 7-day period of simulated marketing. In addition, studies by Yurong et al. [14], where the influence of 1-MCP on changes in the content of phenolic compounds was tested, confirm the loss of total polyphenols and flavonols in apples of the "Jonagold" cultivar. In contrast, Veberic et al. [37] found greater stability of polyphenols in flesh than in apple peel in the case of "Jonagold" and "Golden Delicious". The discrepancies in the results of the studies may result from both the differences between the cultivars and the ripeness of the analyzed fruit, as pointed out by MacLean et al. [17], suggesting that changes in the content of total polyphenols and flavonols during storage and simulated turnover in apples depend on the ripeness of the fruit.

The results of our experiment confirm the effectiveness of the application of 1-methylcyclopropene (1-MCP) during fruit storage. In combinations where the fruit was treated with 1-MCP, this compound positively influenced the phenolic acid content of the apple peel. This effect was more pronounced after storage at ULO for over 10 weeks. A similar relationship was not found in the flesh of apples. The content of flavonols, similarly to phenolic acids, was higher in the peel of fruits treated with 1-MCP than those untreated with this inhibitor, but the content of flavonols in the flesh was stable. Similar results were obtained by Hoang et al. [24] and Kolniak-Ostek et al. [18], who found higher levels of phenolic compounds in fruits treated with 1-MCP after storage, whereas MacLean et al. [17] found no influence of 1-MCP on the level of flavonols.

The antioxidant capacity of both apple peel and flesh in our own studies slightly decreased during the simulated circulation at high temperature, but this trend was only proven in the fruit after 20 weeks of storage at ULO. In studies by Yurong et al. [14] on "Jonagold" apples, a reduction in the antioxidant capacity was also observed during the simulated turnover. In the present experiment, the effect of 1-MCP treatment on the antioxidant capacity was different and depended on the part of the tested fruit. Higher values of this index were found in the flesh of fruits treated with 1-MCP than those that were untreated. In the case of the peel, the effect of 1-MCP was negligible, but it is worth emphasizing that the peel of the fruit treated with 1-MCP showed a slightly reduced antioxidant capacity. This thesis is confirmed by the studies of Hoang et al. [24], which showed a reduction in the antioxidant capacity of apples treated with 1-MCP. Lu et al. [38] and Yurong et al. [14] found an increased antioxidant capacity in the peel of apples treated with 1-MCP after the storage period. On the other hand, Kolniak-Ostek et al. [18] did not observe changes in the antioxidant capacity of apples treated with 1-MCP during storage.

5. Conclusions

The realized experiment, the aim of which was to determine the influence of 1-MCP and period of simulated trade on the content of phenolic compounds and antioxidant capacity of apples, showed that the tested factors had an impact on the health-promoting properties of the fruit. The pro-quality parameters of fruit assessed in the experiment

changed under the influence of the experiment factors, but these changes were more visible in the apple peel than in the flesh. The effect of 1-methylcyclopropene on the content of the tested compounds depended on the part of the tested fruit; i.e., apples treated with 1-MCP were characterized by a higher content of phenolic acids or flavonols in the peel than in the flesh. The content of these compounds in the apple flesh was quite stable, even after 15 days at the temperature of 25 °C. Trade in high-temperature conditions causes a significant loss of antioxidant activity in the flesh of apples, especially in more ripe fruit, i.e., after extended storage in ULO chambers.

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