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PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF UNRIPE APPLES

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Abstract. The aim of this study was to determine the physicochemical indicators and antioxidant activity from the unripe apples obtained after the agricultural thinning operation. Apples of the *Coredana, Golden Rezistent, Reglindis* and *Rewena* varieties harvested in 2020 were studied. Physicochemical indicators were analysed. The quantitative and qualitative determination of organic acids was carried out by the HPLC method, and of carbohydrates by capillary electrophoresis. Determination of antioxidant activity and total polyphenols content was evaluated by spectrophotometric methods. The highest amount of organic acids was obtained in the 45th days after full bloom (DAFB) of harvest, the predominant being malic acid with a value between 15.09±0.02 and 21.64±0.01 g/100g dry weight (DW). Sugars had the highest value in 97th DAFB, fructose being the main one (67.79±0.11 – 75.73±0.10 g/L). Total phenolic content and antioxidant activity showed maximum values at the beginning of fruit harvesting, having 916.67±0.17 – 1316.13±0.21 mg GAE/100g DW and ascorbic acid equivalent antioxidant capacity of 16.94±0.12 – 23.51±0.2 mg AAE/100g DW, respectively. It was concluded that thinned unripe apples represent a natural source of organic acids and carbohydrates, significant amounts of phenolic compounds with antioxidant properties. The study provides information on unripe apples that can be processed and optimally used for food purposes.

Keywords: unripe apple, organic acid, carbohydrate, total polyphenolic content, antioxidant activity.

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Introduction

In the Republic of Moldova, the apple is the predominant fruit tree species, accounting for 60-70% of fruit production, according to the National Bureau of Statistics (NBS) [1], and an important place in exports, according to the Food and Agriculture Organization of the United Nations Statistics (FAOSTAT) [2]. Officially, the total area of orchards in our country in 2022 reached about 57 thousand ha, being cultivated about 40 varieties of apples [1].

Apple trees produce an excess of fruit which negatively affects their commercial value and can lead to a reduced harvest the following year [3-5]. In the early ripening phase, on days 40–45 from the full blooming of trees (DAFB), the physiological fall of undeveloped fruits takes place, and on days 50–65, thinning is carried out [6]. Physiological falls can also occur during the 2 weeks before harvest and are believed to be related to the decrease in incident light and cooler temperatures at the end of the season [7]. Thinning unripe apples balances the amount of fruit left on the trees with the leaf surface that provides the energy for growth and ripening. In plantations, approx. 25–30% of the expected fruit is removed, especially in years with insufficient soil moisture [8-10]. Significant amounts of thinned unripe apples are not used for food. As a rule, they are left in orchards on the ground as waste, representing sources of environmental pollution that cannot be neglected.

According to the literature study, unripe apples contain impressive amounts of valuable substances, such as organic acids, carbohydrates, polyphenols, minerals, *etc*. [11-13]. The content of total organic acids and total polyphenolic content is high in the early ripening phase and decreases during fruit development. Carbohydrates and starch are minimal at the beginning of apple growth, continuously increasing throughout, and starch continuously decreasing after 71st DAFB [14,15]. Malic acid is predominant and constitutes up to 90% of the total content of organic acids in apples [16,17]. Phenolic compounds detected in unripe fruits (~30 DAFB) represent phenolic acids (5-caffeoylquinic acid, 4-*p*-coumaroylquinic acid, chlorogenic acid, caffeic acid, and phenylalanine ester), flavanols, ((+)-catechin, (−)-epicatechin,

and procyanidin B2), flavanones (rutin, quercetin quercetin-3-*O*-galactoside, quercetin-3-*O*-rhamnoside, isoquercetin, and quercetin) and flavonols (phloretin-2′-*O*-xyloglucoside, hydroxyphospholipid monoglycoside, hydroxychrometin diglycoside and hyperoside) [18]. Phenolic compounds determine the antioxidant activity of unripe thinning apples and have a direct proportional relationship with it [19].

Bioactive compounds of unripe apples have many properties such as antioxidant [13,19], antimicrobial [20], antibacterial [21], anti-inflammatory, cardiovascular and hepatic protective [13,22]. These fruits can be used in the food industry, animal farming, agriculture and others. The compounds of the unripe apples can be incorporated into various food products, thus increasing the nutritional value with health benefits [23].

The global problem of environmental degradation is growing. The depletion of resources required the search for new innovative solutions to promote sustainable development [2]. The valorisation of unripe apples is part of the concept of circular economy and sustainable development which are very current and necessary at the global level. This perspective could help reduce the loss of plant raw materials and encourage the transition to sustainable food systems.

The main aim of this study is to determine the physicochemical indices; identify quantitative and qualitative organic acids and carbohydrates, total polyphenolic content and antioxidant activity from the unripe apples of *Coredana, Golden Rezistent, Rewena* and *Reglindis* varieties.

Experimental

Materials

Biological materials

Biological materials for study served unripe apples of *Coredana, Golden Rezistent, Rewena* and *Reglindis* varieties. The fruits were picked in 2020 during development at 45th, 58th, 71st, 84th, and 97th DAFB, from the experimental orchards of the Scientific and Practical Institute of Horticulture and Food Technologies (SPIHFT), Chisinau, Republic of Moldova. This period was between June 4 and July 24, 2020. Images of ripe apples of the studied varieties are presented in Figure 1*(a-d)* [24,25].

Reagents

The Folin-Ciocalteu phenol reagent (2.1 N) was provided by Chem-Lab (Belgium), the 1,1-diphenyl-2-picrylhydrazyl-hydrate DPPH (purity ≥95%) by Sigma-Aldrich (Germany). The standard compounds such as gallic, malic, citric, lactic, tartaric, acetic, ascorbic acids (≥97%), fructose, glucose, sucrose (≥95%); but also solvent acetonitrile, dipicolinic acid (pyridine-2,6-dicarboxylic, DPA) and tetradecyltrimethylammonium bromide (TTAB) were purchased from Sigma-Aldrich (Germany). Ethanol, phenolphthalein, sodium hydroxide, sodium carbonate and potassium dihydrogen phosphate were purchased from Chemapol (Czech Republic). Distilled water was obtained using GFL 2001/4 Water Purification system (Company GFL, Germany). All reagents used were of analytical or chromatographic grade.

Methods

Sample preparation

The unripe apples were manually collected, at a distance of 13 days. They were brought from the orchard in a plastic box with a maximum mass of 10 kg to the research laboratory. The duration from picking from the tree to processing was on average 2–4 hours. The fruits were washed, to remove existing impurities, crushed and then the mass was pressed in a laboratory press (Hotpoint Ariston, Indesit Company), clarified and filtered with the help of the EBA 20 HETTICH centrifuge (Zentrifugen, Germany). The obtained juice presented the samples subjected to research. Analyses were performed for 2–3 days. During this time the researched samples were kept in the refrigerator at a temperature of +5°C. A surplus of squeezed juice was left in the freezer at –18°C for possible further research.

(a) ***(b)*** ***(c)*** ***(d)***

Figure 1. Ripe apples Coredana *(a)*, Golden Rezistent *(b)*, Reglindis *(c)*, Rewena *(d)* varieties [24,25].

Determination of the physical characteristics of the studied apples

Fruit weight was recorded using an electronic balance brand WTC C1 / LW (Radwag, Poland). Measurement of fruit sizes of different apple cultivars was carried out using an electronic caliper (Mityoto, Japonia). Three linear dimensions were measured to determine the average size: length (*L*), width (*W*) and thickness (*T*) [26]. The arithmetic mean diameter (D_a) was calculated using Eq.(1).

$$D_a = (L+W+T)/3 \quad (1)$$

where, *L* is length, equivalent distance of the stem to the calyx, mm;
W is width, the longest dimension perpendicular to *L*, mm;
T is thickness, the longest dimension perpendicular to *L* and *W*, mm.

Physicochemical analysis

Unripe apples were brought to a constant mass at 103±2°C, for determined humidity content, in the laboratory drying oven according to the known method [27]. Total soluble solids were obtained using the electronic refractometer ATAGO PAL-3 (Japan). The titratable acidity was determined by titrating the analysed supernatant with the sodium hydroxide solution in the presence of the phenolphthalein indicator [28], and the ionic acidity with a pH-meter HANNA 211 (Germany) [29]. The relative density was determined using a Density Meter, DMA 58.

Determination of total polyphenolic content

For the determination of total polyphenolic content (TPC), the Folin-Ciocalteau reagent was used by the method described in the literature [30]. In a 50 mL volumetric flask was added 0.5 mL sample and diluted with distilled water (1:10). Then 2.5 mL of Folin-Ciocalteau reagent was pipetted, agitated and 7.5 mL of sodium carbonate solution (Na_2CO_3) 20% was added. The obtained solution was placed in the dark for 30 min at room temperature. After the expiration of the time absorbance of samples was measured on the spectrophotometer UV-Vis SPEKOL 1500 (Analytic Jena, Germany) at wavelength λ= 750 nm in a 1 cm cuvette against the control sample prepared with distilled water instead of the analysed sample. Results were expressed as gallic acid equivalents (in mg GAE/100g DW of product). Determination was according to the calibration curve of the gallic acid standard (0.1-0.50 mg/100 mL; R^2= 09767; y= 0.0227+0.7463x). Average results were obtained from three parallel determinations.

Determination of organic acids by high-performance liquid chromatography (HPLC) analysis

The organic acids in this study were estimated by high performance liquid chromatography. The Agilent Chem Station 7100 (Agilent Technologies, USA) was used. Performance specifications was: real time UV-Visible diode-array detector (190–600 nm); wavelength accuracy: 1 nm; response time: 0.063 to 16 s; light source: prealigned deuterium lamp; baseline noise: <50 μAU (at 2 sec response time); linear dynamic range: 1×10^4 (3×50 μm bubble cell capillary) [31]. The 5 mL of sample was dissolved in distilled water and filtered through a membrane filter (diameter 0.45 μm), then it was injected into the HPLC for analysis. The separation was achieved using a C_{18} column (250×4.6 mm), with a particle size diameter of 5 μm and a guard column (Agilent Technologies, USA). All separations were maintained at 25°C. The detection signal wavelength was 254 nm, and reference wavelength was 210 nm. The mobile phase was composed of KH_2PO_4 buffer solution adjusted to *p*H= 2.8, with a flow rate of 0.7 mL/min. The injection volume of each sample was 10 μL [32]. The retention time of tartaric, citric, malic, succinic, lactic and acetic acids was 3.763 min, 4.078 min, 4.148 min, 4.723 min, 4.807 min, and 5.030 min, respectively. The concentration of organic acids in the samples were expressed as g/L.

Determination of sugars by capillary electrophoresis (CE) analysis

Carbohydrate determination was performed by the capillary electrophoresis (CE) method at the Capel 105M facility (Lumex, Russia). Sample preparation was performed as in the case of HPLC analysis. The separation conditions were as follows: background electrolyte based on dipicolinic acid with the addition of tetradecyltrimethylammonium bromide (TTAB); capillary L_{efect}/L_{total}= 65/75 cm, *ID*= 50 μm; voltage - 25 kV; detection wavelength λ= 230 nm. All separations were maintained at 20°C. The data were processed using the Elforun program. All sample peaks were assigned by comparing retention times with those obtained from standards [33,34]. The concentration of sugars in the samples was expressed as g/L.

Determination of antioxidant activity by reaction with the DPPH• free radical

Antioxidant activity (AA) in the samples was determined using the reaction of DPPH• free radical (spectrophotometric method). A SPEKOL 1500 UV-Vis spectrophotometer (Analytic Jena,

Germany) was used. The decrease in absorbance was measured at 517 nm wavelength (DPPH absorbance maximum), according to the method described in the literature [19]. The results were related to ascorbic acid. Measurements were performed in triplicate. The ascorbic acid equivalent antioxidant capacity (AAEAC), expressed in mg AAE/100g DW, was determined using the ascorbic acid calibration curve (10–45 mg AscAcid/L; R^2= 0.9994; y= 0.4663+0.0032x).

Statistical processing of experimental data

The analysis of the variance of the results was performed using one-way analysis of variance and Student's test. The software application Microsoft Office Excel version 2010 was used for statistical analysis. All determinations were performed in three parallel measurements, with a maximum error of less than 5% (q< 0.05%). The obtained results were expressed as mean ± SD [35].

Results and discussion

Physical indicators of the unripe apples of studying varieties

Unripe apples of the *Coredana, Golden Rezistent, Rewewna* and *Reglindis* varieties harvested between 45th–97th DAFB 2020 year were studied. The physicochemical characteristics and indicators were determined in the fruits. Approximately 5 kg of each variety was collected for analysis on the days established by DAFB. Initially, the physical indicators of the analysed fruits were measured, which showed diameter and mass (Table 1).

Analysis of the physical indicators of the studying unripe apples revealed that their diameter and mass increase essentially during growth and development. The weight of the fruits increased on average by 65%, thus constituting values for varieties 66.3±0.1% *(Coredana)*; 68.9±0.3% *(Golden Resistant)*; 65.3±0.1% *(Reglindis)* and 59.1±0.2% *(Rewena)*. The diameter of the fruits increased, on average, by 44%, with the following values for each variety: *Coredana* - 45.3±0.2%; *Golden Resistant* - 42.3±0.1%; *Reglindis* - 42.9±0.1% and *Rewena* - 45.6±0.2%, respectively. Similar results were recorded in the work done by Mureșan, E.A. *et al.* on the *Golden Delicios, Ionathan* and *Starkrimson* apple varieties, harvested between 7th and 144th days from the full flowering phenophase [36]. Apples show a simple sigmoid curve growth pattern with an initial phase of exponential growth, followed by linear growth, based on an ex-linear equation involving fruit diameter [37].

Table 1

Physical indicators of the unripe apples of *Coredana, Golden Rezistent, Rewewna, Reglindis* varieties.

Variety	*Time, DAFB*	*Arithmetic mean diameter (D_a), mm*	*Weight (m), g*	*Moisture (W), %*	*Juice yield * (η), %*
Coredana	45	29.9±0.1	28.9±0.1	83.20±0.95	25.84±0.11
	58	34.6±0.2	34.5±0.2	84.25±0.83	29.60±0.20
	71	44.8±0.1	45.6±0.1	82.81±0.70	36.35±0.14
	84	47.3±0.1	58.3±0.1	83.07±0.72	43.69±0.15
	97	54.7±0.3	85.8±0.3	81.40±0.24	53.38±0.24
Golden Rezistent	45	30.7±0.1	25.6±0.1	85.13±0.53	27.64±0.21
	58	37.1±0.1	32.3±0.1	84.47±0.07	34.24±0.16
	71	43.4±0.2	47.3±0.2	84.30±0.23	38.65±0.13
	84	49.9±0.2	65.6±0.2	85.62±0.02	46.54±0.16
	97	53.2±0.1	82.4±0.1	84.31±0.32	55.60±0.12
Reglindis	45	30.8±0.2	29.5±0.1	82.40±0.73	25.50±0.16
	58	38.3±0.1	40.1±0.3	81.20±0.91	32.42±0.18
	71	43.8±0.2	52.4±0.2	82.15±0.91	34.04±0.10
	84	50.7±0.3	63.7±0.1	82.30±0.72	44.22±0.21
	97	53.9±0.1	85.0±0.2	81.90±0.09	55.01±0.14
Rewena	45	31.8±0.2	36.2±0.1	84.50±0.07	24.33±0.20
	58	36.7±0.3	42.1±0.1	84.15±0.20	30.15±0.22
	71	44.5±0.1	54.5±0.2	85.05±0.40	37.12±0.15
	84	55.4±0.1	73.6±0.2	84.70±0.08	42.36±0.12
	97	59.5±0.2	88.5±0.1	84.30±0.07	52.55±0.11

*DAFB - days after full bloom, results are expressed as average ± SD (n= 3); * the juice yield after pressing, % of the mass of the raw material.*

During the growth and development of apple fruits, the moisture content varied between 81.20±0.91% and 85.62±0.02% and no significant differences were observed between the tested varieties (Table 1). Humidity is an important parameter to determine, because it influences the yield of apple juice. Zheng, H.Z. *et al.* and Mureșan, E.A. *et al.* reported similar results after studying *Fuji* (82.38–87.64%), *Ionathan* (83.62–87.71%), *Starkrimson* (83.39–87.87%) and *Golden Delicious* (80.38–87.82%) apple varieties. The fruits were investigated between 25th–105th DAFB and 7th–144th DAFB, respectively [36,38].

The yield of juice squeezed from apples was on average between 25 and 55%, depending on the variety and the date of harvest. The highest yield was obtained from the *Golden Rezistent* and *Reglindis* apple varieties with the following results: 27.64–55.60% and 25.50–55.01%, respectively. Then follows *Coredana* with 25.84–53.38% and *Rewena* with 24.33–52.55%.

Physicochemical indicators of the unripe apple juice

Juices were obtained from the unripe apples of the studied varieties by pressing. In them, such physicochemical indicators as titratable acidity, total soluble solid (TSS), density and pH were determined. The analysis of the obtained data served as a general picture and the identification of the fields of valorisation and processing of thinned unripe apples. The results of all indicators are presented in Figure 2*(a-d)*.

The titratable acidity in the studied apples decreased during fruit growth. The indicator values were recorded between 1.50 and 2.14% for the *Coredana* variety; 1.94 and 2.80% for the *Golden Resistant* variety; 2.09 and 2.71% for the *Reglindis* variety and between 1.74 and 2.65% for the *Rewena* variety. The highest acidity concentrations were recorded in fruits harvested at 45th DAFB, decreasing slightly during development in all studied cultivars. The slight decrease could be due to the degradation of citric acid, the transformation of acids during respiration, their conversion to sugars and their subsequent use in the metabolic process in the fruit [14,39]. At the same time, the processes involved in the metabolism and accumulation of malic and citric acids in mesocarp cells are under both genetic and environmental control [40].

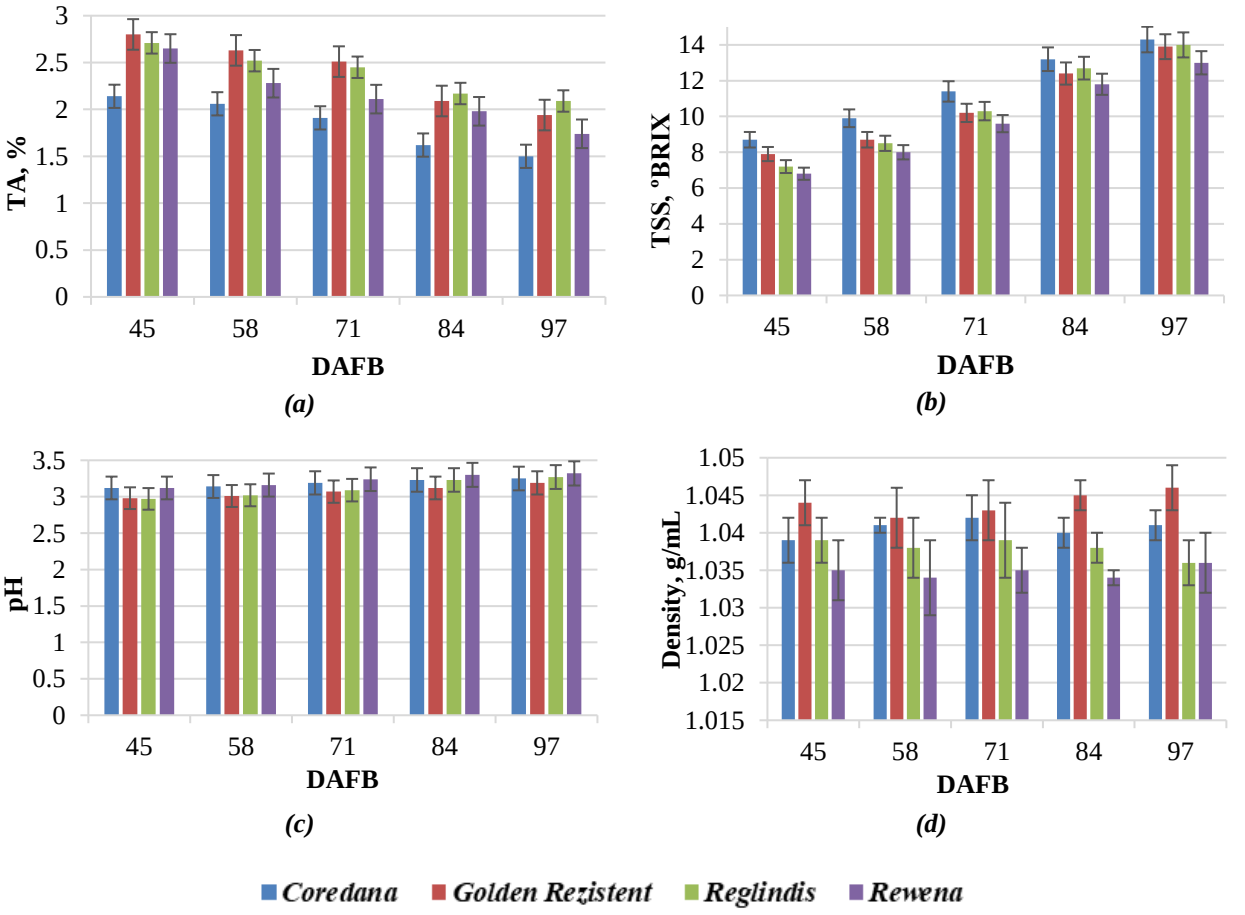

Figure 2. The evolution of the physicochemical indicators in the four unripe apple varieties during development: TA – titratable acidity, exprimated % malic acid *(a)*, TSS – total solubil solids *(b)*, pH *(c)*, density *(d)*. DAFB-days after full bloom.

The TSS content increased considerably with the development of the studied fruits. The highest values of this index were recorded for all apple varieties towards 97th DAFB. A sharp increase in TSS was observed around the 84th DAFB. This fact can be explained by starch hydrolysis in unripe apples at the given stage. Some researchers have shown that starch accumulation occurs between the 35th and 85th DAFB, reaching a maxim around 85th DAFB [36,38,41]. In *Coredana* apples, the amount of TSS (^{o}Brix) was between 8.70 and 14.30; *Golden Resistant* - between 7.90 and 13.90; *Rewena* - between 7.20 and 14.00; *Reglindis* - between 6.80 and 13.01.

The pH of the apple depends mainly on the organic acid contained. The *p*H values of the apple pulp do not change essentially, however, a small increase of this indicator was recorded during the growth and development of the fruit. The values of all fruits were between 2.97 and 3.32. Zheng, H.Z. *et al.* reported similar results in their studies for the *Fuji* variety [38].

The density of all studied samples, similarly, did not have essential changes during development, presenting values between 1.034 and 1.046 g/mL. Wicklund, T. *et al.* studied 15 apple varieties from Norway and it was established that the density of the studied apple juices was between 1034 and 1060 g/L [42].

Determination of organic acids by HPLC analysis

The amount of malic, citric, succinic, acetic, lactic and tartaric acids was determined in the studied samples by the method of HPLC. According to the results obtained (Figure 3), it can be concluded that the unripe apples of all 4 varieties studied, depending on the harvest time, contain impressive amounts of organic acids.

Malic acid was predominant from the total organic acids detected in all the samples studied, with values between about 95% and 98%. Many researchers have shown in their studies that apples predominantly accumulate malic acid (about 90%) compared to other organic acids [40,43,44]. In apples during ripening, the content of malic acid decreased in the *Coredana* variety from 15.09 g/100g dry weight (DW), harvested at 45th DAFB, to 9.66 g/100g DW, towards 97th DAFB. Similarly, it decreased in the varieties *Golden Rezistent* (from 24.07 to 10.98 g/100g DW) and *Reglindis* (from 21.64 to 11.33 g/100g DW), followed by *Rewena* (from 21.40 to 10.76 g/100g DW) (Figure 3). Malic acid was detected in all *Malus* species, ranging from 1.72 to 29.27 mg/g fresh weight (FW), with an average of 8.90 mg/g FW [44].

Citric acid was predominantly detected in several wild apple species, with content ranging from undetectable to 24.24 mg/g FW, according to literature data [44]. In the studied samples it was between 0.65 and 2.02% from the total organic acids detected, and had average values or all 4 varieties of apples between 0.09 and 0.28 g/100g DW. Its highest levels were observed in *Rewena* apples during development from 45th DAFB to 97th DAFB with a concentration of 0.25–0.23 g/100g DW. The low level of citric acid is primarily controlled by organic acid metabolism [40].

The apple variety with the highest concentration of succinic acid was *Reglindis* with values between 0.03 and 0.11 g/100g DW, and the average value for all varieties during the research was 0.02 and 0.11 g/100g DW. This quantity of succinic acid constitutes 0.13–0.56% of the total organic acids detected in the studied samples. Succinic acid, like malic and citric acids, has significant amounts in unripe apples compared to ripe ones [43,45]. Celik, F. *et al.* demonstrated that the succinic acid content in apples varies between 0.144 and 0.511 mg/100 mL [46].

On the surface of the fruits in small quantities, in the case of healthy fruits, or in abundance, in the case of spoiled ones, there are acetic and lactic bacteria that produce the corresponding acids [47]. In the analysed apples, the amounts of these acids were very low, with 0.27–0.95% of acetic acid and 0.49–0.98% of lactic acid, from the total organic acids detected. According to the results obtained, their average values for all apple varieties represented 0.07–0.13 g/100g DW of acetic acid and 0.05–0.14 g/100g DW of lactic acid, respectively.

During the growth and development of the apple, the levels of acid concentrations decrease, the exception being tartaric acid which accumulates in an advanced stage of maturity [43,45]. The amount of tartaric acid in the analysed fruits was 0.60–1.15% from the total organic acids detected, which represented on average values between 0.12–0.17 g/100g DW. In the study carried out by Celik, F. *et al.* the content of tartaric acid in ripe apples varies on average from 0.075 to 0.214 mg/100 mL [46].

According to the obtained results, it can be observed that the maximum amounts of organic acids detected in the studied unripe apples were on day 45th DAFB of harvesting, the exception being tartaric acid with a maximum concentration at 97th DAFB. The results are

similar to the claims of other researchers, who studied the significant variation of organic acid components detected in cultivated apple fruits and showed that most of the malic acid (the main organic acid) is located in the vacuole of the parenchyma cells [48]. Its concentration shows a developmental pattern that reaches its maximum up to 6 weeks (~45 days) after blooming, followed by a continuous decrease until fruit harvest [45].

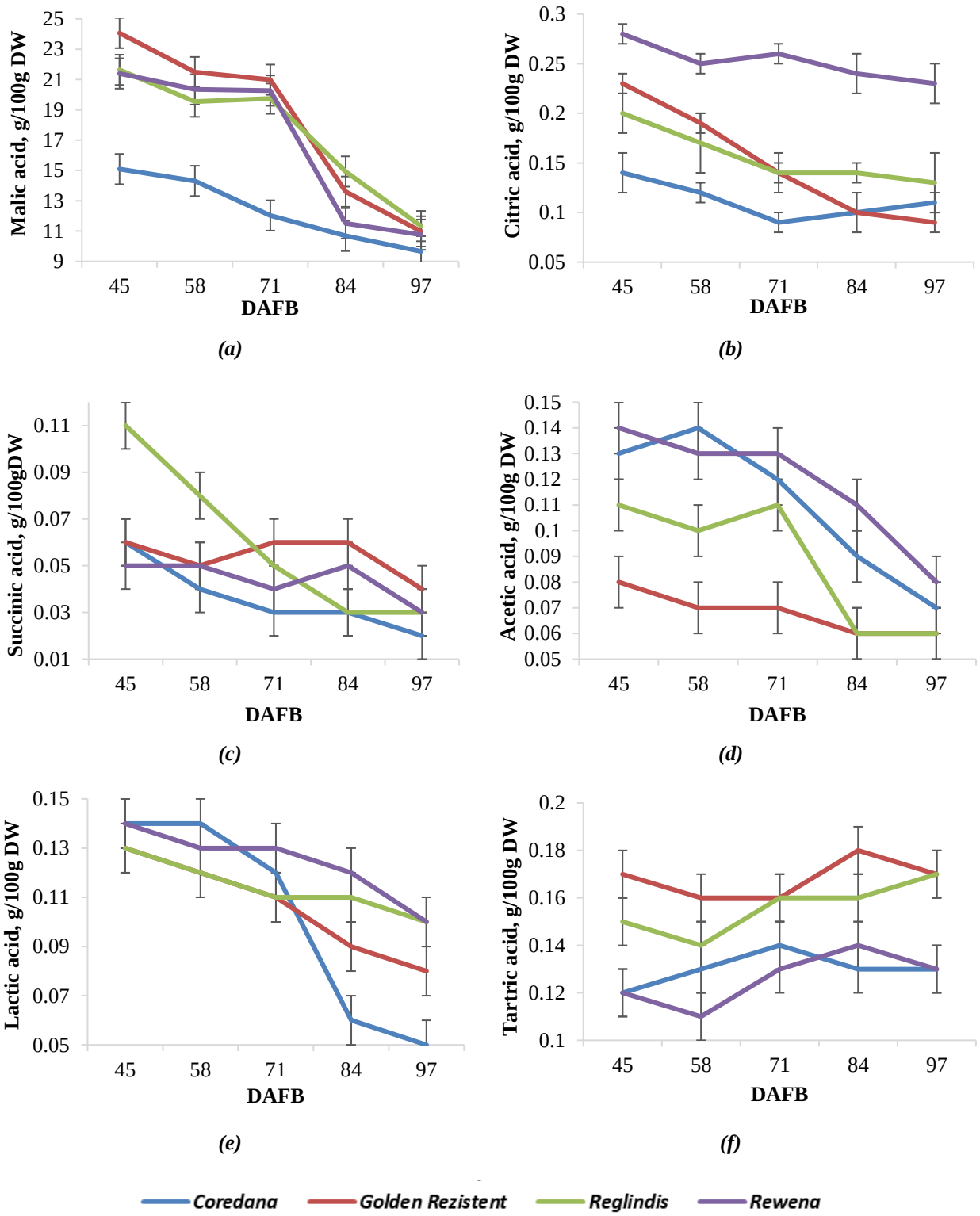

Figure 3. The content of organic acids determined in apples of the *Coredana, Golden Rezistent, Reglindis* and *Rewena* varieties during development, expressed in g/100g DW: malic acid *(a)*, citric acid *(b)*, succinic acid *(c)*, acetic acid *(d)*, lactic acid *(e)*, tartric acid *(f)*. DAFB-days after full bloom.

Capillary electrophoresis analysis of sugars

The content of simple carbohydrates in the harvested apples were analysed individually and are presented in Figure 4.

The amount of fructose and glucose had continuously increased in all studied samples, reaching maximum on 97th DAFB. Sucrose content increased until around 71st DAFB, then continuously decreased (Figure 4). The fructose content was from 65.68 to 74.36% of the total carbohydrates determined (a maximum amount 67.79–75.73 g/L), and the glucose content was twice as low and showed 25.62–34.68% (maximum 23.36–29.15 g/L). Sucrose was detected in small amounts, constituting 0.04–0.48% (content being between 0.03 and 0.24 g/L). Apples harvested at 84th DAFB in all 4 cultivars studied showed a sudden increase in the amount of fructose and a sudden decrease in sucrose, followed by a slow increase. The sudden changes may be due to the hydrolysis of the starch contained in unripe apples and reaching a maximum amount around this period [38] and the fact that more than half of the sucrose is converted to fructose [45,49]. The concentration of fructose increased rapidly from 4 to 12 weeks after bloom, and then remained unchanged to fruit harvest. Sucrose did not show rapid accumulation until 6–8 weeks after bloom, but increased all the way to fruit harvest [40,44].

Total polyphenolic content and antioxidant activity

The relationship between total polyphenolic content (TPC) and antioxidant activity (AA) in thinned apples is directly proportional. AA of apples is mostly determined by TPC [50-52]. In the analysed samples of unripe apples, the amount of TPC and AA were determined, and the results were presented in Figure 5.

TPC concentrations in all samples are quite significant (Figure 5*(a)*). The lowest TPC level (mg GAE/100g DW) was 306.45–916.67 in apples of the *Coredana* variety, while the highest values were detected in the fruits of the *Rewena* variety: 547.77–1316.13. *Golden Rezistent* and *Reglindis* varieties apples had similar amounts of polyphenols (mg GAE/100g DW): 490.76–1331.54 and 453.04–1096.59, respectively. Geleta, B.T. *et al.* investigated 10 varieties of thinned unripe apples and obtained TPC in the range of 8.97–81.4 mg GAE/g [13].

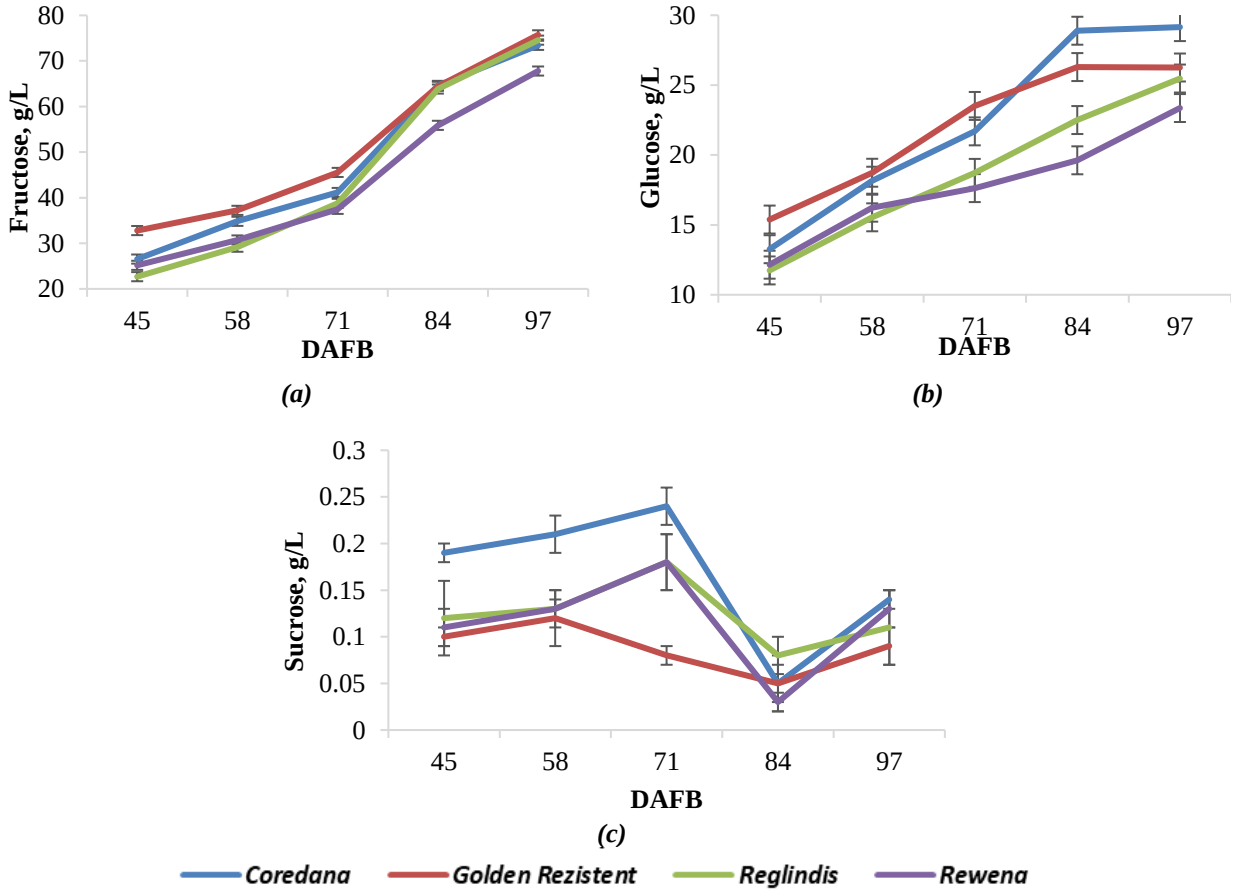

Figure 4. The content of carbohydrates determined in apples of the *Coredana, Golden Rezistent, Reglindis* and *Rewena* varieties during development, expressed in g/L: fructose *(a)*, glucose *(b)*, sucrose *(c)*. DAFB-days after full bloom.

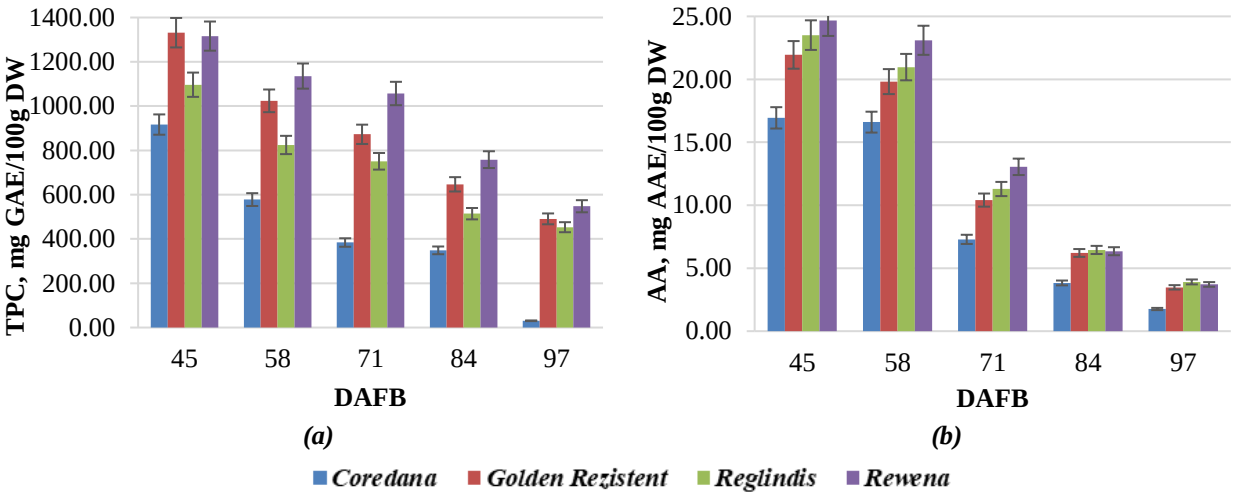

Figure 5. Change in total polyphenolic content (TPC) *(a)* and antioxidant activity (AA) determined *(b)* in apples *Coredana, Golden Rezistent, Reglindis* and *Rewena* varieties during development. DAFB-days after full bloom.

Apples harvested in the period 45th and 58th DAFB are characterized by higher AA values (mg AAE/100g DW), constituting between 16.60 and 16.94 for the *Coredana* variety; 19.82 and 21.94 for the *Golden Resistant* variety; 20.94 and 23.51 for the *Reglindis* variety; 23.10 and 24.68 for the *Rewena* variety. Around the 71st day of harvest, a continuous sharp decrease in AA was observed until the 97th DAFB (Figure 5*(b)*). Wojdyło, A. *et al.* in his study on apples during the development period from 60 DAFB to 140 DAFB showed that AA in three apple varieties varied between 3.6 and 46.1 mM Trolox/g DW [19].

Accumulation levels of abundant secondary metabolites, especially TPC, rapidly decrease in apple fruit during development from 27th to 84th DAFB [43]. During the given period, the biosynthesis of secondary metabolites and the stage of cell expansion occur, which leads to a high level of metabolic activity in apples [38,45]. The high levels of antioxidant capacity are explained by the high amount of biologically active compounds with an antioxidant character, especially phenolic compounds (Figure 5). Unripe apples are a good source of bioactive compounds, which is reflected in the high values of antioxidant properties [19].

Conclusions

The conducted study demonstrated that thinned unripe apples represent a cheap and accessible source of valuable natural nutrients such as organic acids, carbohydrates, polyphenolic compounds with antioxidant properties. The fruits of the varieties *Coredana, Golden Rezient, Reglindis* and *Rewena*, harvested in June-July 2020 (between 45th and 97th DAFB), were investigated.

The titratable acidity in the studied apples was decreasing during fruit growth. The quantitative and qualitative determination of organic acids showed that malic acid is predominant (95–98%) with values for *Coredana* 9.66–15.09 g/100g DW, *Golden Resistent* 10.98–24.07 g/100g DW, *Reglindis* 11.33–21.64 g/100g DW and *Rewena* 10.76–21.40 g/100g DW. Citric, succinic, acetic, lactic and tartaric acids had very small amounts.

The amount of carbohydrates tended to increase in the analysed fruits during development. The main carbohydrate detected was fructose (65.68–74.36%), and the glucose content was twice as low (25.62–34.68%). The amounts of fructose and glucose had the following values: for *Coredana* 34.82–73.42 g/L and 18.16–29.15 g/L, *Golden Resistant* 37.23–75.73 g/L and 18.73–26.26 g/L, *Reglindis* 29.14–74.56 g/L and 15.54–25.47 g/L, *Rewena* 30.73–67.79 g/L and 12.15–23.36 g/L, respectively. Sucrose was detected as traces (0.04–0.48%).

Unripe apple fruits have a high content of total phenolics (916.67–1316.13 mg GAE/100g DW) with significant antioxidant properties (with AAEAC values of 16.94–23.51 mg AAE/100g DW). They had a tendency to decrease during the ripening of the apples at the times and varieties studied.

Unripe thinned apples represent an accessible source of natural biological compounds, which can be recovered and used as natural additives in the food industry for the production of natural and healthy foods.

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