

Article

Biochemical and Volatile Compound Variation in Apple (*Malus domestica*) Cultivars According to Fruit Size: Implications for Quality and Breeding

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Abstract

Apple fruit size affects market value, yet its impact on biochemical and sensory traits is poorly understood. This study provides the first comprehensive metabolic profiling of peel and flesh across five cultivars, including red-fleshed ‘Baya Marisa’ and four white-fleshed cultivars (‘Opal’, ‘Red Boskoop’, ‘Crown Prince Rudolf’, and ‘Topaz’), in two size groups: large (>70 mm, Class I) and small (55–70 mm, Class II). Sugars and organic acids varied by cultivar but not consistently by size. White-fleshed small apples had higher flesh phenolics, suggesting a dilution effect, while ‘Baya Marisa’ showed no size-related phenolic differences, indicating potential genetic influence. VOCs were mainly aldehydes, with cultivar-specific differences outweighing size effects. Fruit maturity and controlled-atmosphere storage likely limited ester production. These findings demonstrate that fruit size influences certain biochemical traits in a cultivar-dependent manner. This study’s novelty lies in combining tissue-specific metabolite profiling with size comparisons across multiple cultivars, providing practical insights for breeders, nutritionists, and the fruit industry. This work supports size-specific quality assessment and valorization of smaller apples for fresh consumption and processing, challenging conventional market classifications based solely on size.

Keywords: apple cultivars; aroma profile; fruit quality; fruit size; organic acids; phenolic compounds; sugars; volatile organic compounds

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1. Introduction

Several parameters are commonly used to assess fruit quality, including external characteristics such as appearance (color, size, shape, and the presence of physiological or pathological disorders), texture, flavor, and, more recently, nutritional properties [1]. In addition to nutritional composition, non-nutritional properties, particularly antioxidant activity and phenolic compound content, are also important indicators of fruit quality [2].

Apples are typically classified into three commercial categories: Class I, Class II, and apples designated for industrial processing. These classifications are based on standardized quality criteria following postharvest preparation and packaging. Class I apples are considered high quality and are evaluated based on attributes such as fruit size, surface coloration (dependent on cultivar-specific color group), firmness, soluble solid content (°Brix), and the

absence of significant defects. Class II apples meet the minimum requirements for marketability but may exhibit minor defects in shape, size, or coloration [3].

Consumers often rely on visual cues when assessing fruit quality, and size has consistently emerged as one of the most influential attributes. In the case of apples, research shows that larger fruits are frequently perceived as superior, both in terms of appearance and market value. Studies demonstrate that larger apples are often judged as more appealing, while smaller apples tend to be associated with lower quality. This perception persists even though sensory evaluations sometimes reveal that smaller apples can have stronger flavor, better juiciness, and a more concentrated taste. The contrast between market preference for larger fruit and the actual eating quality of smaller apples highlights an important tension in consumer perception: while visual appearance drives initial judgments, sensory attributes ultimately shape satisfaction. Understanding why smaller apples are undervalued is therefore essential, not only for growers and marketers but also for aligning consumer expectations with genuine quality [1].

Fruit size may influence the concentration of phenolic compounds due to a dilution effect, where larger fruit size can result in reduced concentrations of certain nutrients, sugars, organic acids, and phenolics [1]. The profile of phenolic compounds in apples is highly variable rather than uniform. Their levels are influenced by several factors, including cultivar type, stage of fruit maturity, cultivation and growth conditions, harvest timing, postharvest storage, and exposure to pathogens. In addition, the peel and flesh of apples differ considerably in their chemical composition [4]. Apples are recognized as a rich source of phenolic compounds, which are broadly categorized into flavonoids and nonflavonoids based on their structural characteristics [5]. Flavonoids in apples include several subclasses such as flavonols, flavanols, dihydrochalcones, and anthocyanins. Among phenolic acids, chlorogenic acid is the predominant compound found in apple fruits [6].

Apple quality is commonly assessed through characteristics such as visual appeal, texture, firmness, and the absence of physiological or pathological defects. In recent years, however, the definition of quality has expanded, with increasing emphasis placed on sensory properties to better meet consumer preferences. Among these attributes, flavor is particularly critical and serves as a defining trait of apples. Flavor results from the combined perception of taste and aroma: taste is determined by the balance of sugars and organic acids, whereas aroma is a complex of volatile compounds, the composition of which is not only species-specific but often cultivar-dependent [7]. Taste is primarily influenced by the balance of sugars and organic acids, whereas aroma results from a complex mixture of volatile organic compounds (VOCs), which vary by cultivar [8]. More than 300 volatile compounds are found in apples, where the major classes of aroma-related VOCs include esters, alcohols, alkenes, ketones, and aldehydes, where only a few can contribute to fruit aroma [9]. In unripe apples, aldehydes dominate the volatile profile, but their concentration declines during ripening, while esters and alcohols increase, becoming the major contributors to aroma in mature fruit [8]. When odor thresholds are considered, several compounds emerge as key contributors to apple aroma, including ethyl 2-methylbutyrate, hexanal, 1-hexanol, E-2-nonenal, and linalool. Moreover, both the levels of individual volatiles and the overall odor activity values (OAVs) were found to vary across cultivars and between growing seasons, suggesting that seasonal conditions play a significant role in shaping the volatile profile of certain apple varieties [10]. Aldehydes in apples are mainly generated through the oxidative breakdown of unsaturated fatty acids [11]. Subsequent reduction in aldehydes produces alcohols, which serve as precursors in the final step of ester synthesis [12]. In ripe apples, alcohols comprise approximately 6–16% of the total volatile content, while esters account for 80–98% [13,14].

Apple aroma composition is influenced by multiple factors, with cultivar being a primary determinant. The stage of fruit maturity also plays a key role in shaping the levels of volatile compounds. Additionally, pre-harvest management, gene expression, and environmental

conditions—such as light exposure, irrigation, and the use of agrochemicals—can modify aroma production. Postharvest practices, including storage methods, atmospheric conditions, and chemical treatments, further impact both the concentration and composition of aroma compounds. Moreover, the volatile profile varies between intact fruit and those that are physically damaged [9].

The objective of this study was to evaluate and compare the composition of organic acids, sugars, phenolic compounds, and volatile compounds between large-sized apples (diameter > 70 mm; Group I) and small-sized apples (diameter < 70 mm; Group II) of the same cultivars. Apples with a diameter below 70 mm are typically classified as Class II and considered to have lower market value. Both peel and flesh tissues were analyzed separately. The cultivars included in this study were the red-fleshed ‘Baya Marisa’ (‘BM’) and the white-fleshed ‘Opal’ (‘OP’), ‘Red Boskoop’ (‘RB’), ‘Crown Prince Rudolf’ (‘CAR’), and ‘Topaz’ (‘TO’). To the best of our knowledge, no previous studies have directly compared primary and secondary metabolite profiles, along with volatile compound compositions, between different fruit size categories within the same apple cultivar. Previous research has primarily focused on comparisons among different cultivars. The results of this study aim to clarify whether smaller apples, typically considered lower in quality due to their commercial classification, also differ in their biochemical composition. These findings are expected to provide valuable insights for nutritionists, food scientists, and the fruit processing industry, with implications for the valorization of lower-grade apples.

2. Materials and Methods

2.1. Plant Material

For the experiment, five apple cultivars were obtained: red-fleshed and red-skinned ‘Baya Marisa’, white-fleshed and yellow-skinned ‘Opal’, white-fleshed and red/orange whitish yellow-skinned ‘Red Boskoop’, ‘Crown Prince Rudolf’, and ‘Topaz’. The fruit samples were harvested in their harvest windows. ‘Crown Prince Rudolf’ on 18 September 2023, ‘Baya Marisa’ on 21 September 2023, ‘Opal’ on 24 September 2023, ‘Red Boskoop’ on 1 October 2023, and ‘Topaz’ on 28 October 2023. All cultivars were grown on M9 rootstock and planted at a spacing of 3.2 m × 0.9 m. At the time of harvest, they were in their seventh growing season, with orchard rows arranged in a north–south direction for the best light exposure. The tree health was maintained with standard integrated pest management. Following harvesting, the apples were transported into cold storage (3 °C) in the Department of Agronomy, Biotechnical Faculty, University of Ljubljana (Slovenia) for further analysis.

A total of 50 apples (25, 70+ mm, and 25, 55–70 mm) were collected from the cold storage and analyzed at the laboratory of the Department of Agronomy, Biotechnical Faculty, University of Ljubljana (Slovenia). Apple fruits were washed and visually inspected to ensure the absence of pathological and physiological disorders, which could influence the experimental results. Ten apples per cultivar (five 70+ mm and five 55–70 mm) were chosen as biological replicates. Color, firmness, soluble solid content, and starch were measured. The skin was then peeled and the cores were removed, as skin and flesh were analyzed separately. The flesh and skin were cut into small pieces, frozen with liquid nitrogen, ground, placed in tubes, and stored in a freezer (−20 °C) for further analysis. Two tubes, one for the skin and one for the flesh, were used per apple.

2.2. Color

The color of both the sun-exposed side and the shaded side was measured for all cultivars, Class I and Class II apples. The parameters were measured in the CIELAB color space using a colorimeter, CR-10 Chroma (Minolta, Osaka, Japan). The color parameters included a^* values, indicating the red–green axis (positive is red, negative is green), b^*

values, representing the yellow–blue axis (positive is yellow, negative is blue), L^* values, indicating the lightness (0 is black, 100 is white), h° values, representing the color in degrees (0° corresponds to red, 90° corresponds to yellow, 180° corresponds to green, and 270° corresponds to blue), and C^* values, indicating the chroma (the higher the value, the more intense the color).

2.3. Fruit Diameter

All apple cultivars were graded according to size (Digital caliper, Model 2254) and weight (70+ mm and 55–70 mm). Surface color, soluble solid content, and flesh firmness were measured. Cultivars ‘Red Boskoop’, ‘Topaz’, and ‘Crown Prince Rudolf’ are classified as color group B, where 1/3 of the total surface needs to be a red-colored mix. Apple cultivar ‘Baya Marisa’ belongs to color group A and is expected to have 1/2 of the total surface colored red. Cultivar ‘Opal’ is classified in color group D, where no minimal color requirements are needed. The fruits of caliber 70+ mm without any visible defects were chosen and compared to apple fruits with diameters ranging from 55 to 70 mm. For the purpose of this study, apples with any physical or pathological defects, which could influence the results of this study, were excluded. Determination of the appropriate apple fruits for this study was based on fruit color coverage, fruit size, and weight.

2.4. Flesh Firmness and Soluble Solid Content

Flesh firmness was evaluated using a digital penetrometer (T.R. Turoni, Forli, Italy) equipped with an 11 mm piston, with results recorded in kg/cm². The soluble solid content was determined with a digital refractometer (Milwaukee Digital Brix Refractometer MA871, Rocky Mount, NC, USA).

2.5. Extraction of Sugars, Organic Acids, and Phenolic Compounds

Extraction of sugars, organic acids, and phenolic compounds was performed according to previous protocols [15–18]. Fifty apples, ten per cultivar (five 70+ mm and five 55–70 mm) were used for extraction. There were 5 biological replicates per treatment. For the extraction of phenolic compounds, 2.5 g of skin and 5 g of flesh was extracted with 5 mL of 80% acidified methanol (3% formic acid) (MeOH; Sigma-Aldrich, Steiheim, Germany), followed by 60 min of a cooled ultrasonic bath (0°C) and centrifugation for 10 min at $10,000\times g$ at 4°C (Eppendorf Centrifuge 5810 R, Eppendorf, Hamburg, Germany). The supernatant was filtered through $0.20\ \mu\text{m}$ polyamide filters (Macherey-Nagel, Duren, Germany). Extracts were then stored at -20°C until HPLC analysis.

For the extraction of sugars and organic acids, the skin and the flesh were also separated. Briefly, 1 g of the skin and flesh was extracted with 5 mL of bi-distilled water. The sample was then shaken at room temperature for 30 min, followed by centrifugation for 10 min at $10,000\times g$ at 4°C (Eppendorf Centrifuge 5810 R, Germany). For filtration of the supernatant, $0.20\ \mu\text{m}$ cellulose filters (Macherey-Nagel, Germany) were used.

For the determination of organic acids, a Vanquish HPLC (ThermoScientific, Waltham, MA, USA) was used. The analysis conditions were as follows: column (Rezex ROA-Organic acid H+ 8% (150 mm $\times$ 7.8 mm), Phenomenex, CA, USA) with a flow rate of 0.6 mL/min at 65°C for 15 min, where the mobile phase was a 4 mM sulfuric acid solution in bi-distilled water. The injection volume was 20 μL . At 210 nm, the response of the samples was measured with a UV detector. The identification of organic acids was made with analytical standards for fumaric, malic, and citric acid (Fluka Chemie, Buchs, Switzerland) and shikimic acid (Sigma-Aldrich, Steinheim, Steinheim am Albuch, Germany).

The sugar content was analyzed by Vanquish HPLC (ThermoScientific, Waltham, MA, USA). Rezex RCM-monosaccharide Ca+ 2% (300 mm $\times$ 7.8 mm) from Phenomenex (CA, USA) was used as column. The conditions were as follows: flow rate of 0.8 mL/min

at a temperature of 85 °C. The mobile phase was bi-distilled water. The injection volume for the sample was 20 µL. The response of individual sugars was detected using a refractive index (RI) detector (Refractomax 520, IDEX Health and Science KK 5-8-6, Kawaguchi, Japan). Analytical standards for glucose, sucrose, fructose, and sorbitol (Fluka Chemie, Buchs, Switzerland) were used for the identification of individual sugars.

Quantification of phenolic compounds was performed on HPLC (ThermoScientific, USA). For anthocyanins, the diode array detector was set at 530 nm, at 350 nm for flavonols, and at 280 nm for other phenolic compounds. The conditions were the same as previously described in protocols [17,18]. The injection volume was 20 µL. Recorded chromatograms ranged from 200 nm to 600 nm. For the separation of phenolic compounds, a C18 column (Gemini; 150 × 4.60 mm, 3 µ; Phenomenex, Torrance, CA, USA) at 25 °C was used. The identification of phenolic compounds was made with tandem mass spectrometry (MS/MS; LCQ Deca XP MAX; Thermo Scientific, Waltham, MA, USA). Heated electrospray ionization operating in positive ion mode was used for the detection of the anthocyanins, while the remaining compounds were identified in a negative ion mode. Complete MS scans ranging from m/z 50 to 2000 were obtained for analysis. Xcalibur 2.2 software (Thermo Fischer Scientific Institute, Waltham, MA, USA) was used for data acquisition. The quantification and identification of phenolic compounds was performed using external standards, when available. Unknown compounds were identified with MS fragmentation and the literature data, where similar standards for the quantifications were used.

2.6. Extraction and Determination of Volatile Organic Compounds

The profile of volatile compounds was obtained using the gas chromatography analysis (HS-GC-MS), according to previous protocols [19], with some minor modifications. Frozen samples of skin and flesh were ground to fine powder using liquid nitrogen and an analytical mill (IKA A11 basic, Staufen, Germany). The obtained powder from the skin and the flesh (5 g each) was stored in 20 mL vials, with the addition of 10 µL internal standard (IS: 3-Nonanone, 1:8000, 0.09 mg/mL in acetonitrile). The vials were closed with screw caps with a PTFE–silicon septum and then transferred to a Shimadzu AOC-20s autosampler, where the incubation was made at 50 °C for 10 min with consistent shaking at 250 rpm. A 1000 µL sample from the headspace was injected into the injection port at 250 °C using a 1:10 split ratio for 0.4 min with an injection flow rate of 25 mL/min. A Shimadzu GC-MS QP2020 gas chromatograph, connected with a Single Quadrupole Mass Spectrometer (MS) equipped with an EI detector, was utilized. For the separation of volatile compounds, a ZB-wax PLUS capillary column (30 m × 0.25 mm, 0.5 µm film thickness) was used. The helium with a flow rate of 4 mL/min was the carrier gas. The temperature program was first set and held at 45 °C for 3 min, then raised to 150 °C with a 4 °C/min rate, and then raised again to 220 °C at 10 °C/min. Finally, it was held for 5 min at 220 °C. The MS ion source and the interface had the temperature set at 240 °C, with a scan rate of 2.0 scans/s. The ionization energy was at 70 eV, while the mass scan range was set at 50–500 m/z . Volatile compounds were identified by comparing their spectra with commercial libraries (NIST 11 and FFNSC 4) and semi-quantified based on each compound and internal standard peak areas, as well as the internal standard and sample weights.

2.7. Chemicals

The standards applied during the analysis included chlorogenic acid, cryptochlorogenic acid, quercetin-3-O-rutinoside, and shikimic acid, all of which were sourced from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Additional standards—such as *p*-coumaric acid, ferulic acid, procyanidin B1, cyanidin-3-O-galactoside, (–)-epicatechin, caffeic acid, quercetin-3-O-glucoside, quercetin-3-O-galactoside, quercetin-3-O-rhamnoside,

phloridzin, citric acid, malic acid, fumaric acid, fructose, glucose, sorbitol, and sucrose—were obtained from Fluka Chemie GmbH (Buchs, Switzerland). Further standards including cyanidin-3-*O*-arabinoside, quercetin-3-*O*-arabinofuranoside, quercetin-3-*O*-xyloside, and quercetin-3-*O*-arabinopyranoside were provided by Apin Chemicals (Abingdon, UK).

For all stages of sample preparation and analysis, bi-distilled water purified using a Milli-Q purification system (Millipore, Bedford, MA, USA) was utilized. The solvents employed in the preparation of mobile phases for mass spectrometry analysis—namely formic acid and acetonitrile—were of HPLC-MS grade and supplied by Fluka Chemie GmbH (Buchs, Switzerland). Methanol, used for extraction procedures, was of HPLC grade and sourced from Sigma-Aldrich (Steinheim, Germany). Metaphosphoric acid, applied for the extraction of ascorbic acid, was also purchased from Sigma-Aldrich (Steinheim, Germany).

2.8. Statistical Analysis

Statistical analysis was made with R commander ×64 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). The results are expressed as means ± standard error. For statistically significant differences, a *t*-test was used. To calculate the significance of difference, the confidence level was 95%.

3. Results

3.1. Physical Parameters and Starch

Physical parameters including diameter (mm), weight (g), flesh firmness (kg/cm²), soluble solid content (°Brix) (SSC), and starch index (iodine starch test) are summarized in Table 1. The classification of apple fruits into two size categories (Group I: >70 mm; Group II: 55–70 mm) was confirmed by statistically significant differences (*p* < 0.05) in both diameter and weight within each cultivar, validating the accuracy of size-based sorting. In contrast, no significant differences (*p* > 0.05) were observed in flesh firmness, SSC, or the starch index across size groups for all cultivars. This uniformity in key physiological maturity parameters ensured that fruit size was the primary variable influencing biochemical and volatile profiles, thereby reinforcing the reliability of subsequent analyses.

Table 1. Physical parameters and starch test on apple fruits of two different sizes (Groups I and II) of five different apple cultivars ('BM', 'CAR', 'OP', 'RB', and 'TO').

Cultivar	Diameter (mm)		Weight (g)	Flesh Firmness (kg/cm ²)	SS Content (°Brix)	Starch
	Horizontal	Vertical				
'BM' I	78.52 ± 1.19 *	85.44 ± 1.53 *	257.58 ± 9.38 *	5.65 ± 0.18	15.47 ± 0.19	n.d.
'BM' II	60.55 ± 0.91	69.13 ± 0.64	136.89 ± 3.25	6.79 ± 0.24	14.13 ± 0.29	n.d.
'CAR' I	62.96 ± 1.17 *	78.78 ± 1.02 *	177.19 ± 5.88 *	7.04 ± 0.15	14.46 ± 0.40	3.8 ± 0.22
'CAR' II	48.85 ± 0.76	60.46 ± 0.44	82.18 ± 1.7	7.98 ± 0.23	14.41 ± 0.62	4.3 ± 0.17
'OP' I	70.38 ± 1.36 *	78.29 ± 0.82 *	206.30 ± 5.54 *	7.04 ± 0.13	14.33 ± 0.19	4.00 ± 0.75
'OP' II	57.72 ± 0.82	65.94 ± 0.55	121.18 ± 2.58	7.33 ± 0.14	15.16 ± 0.34	4.00 ± 0.00
'RB' I	67.49 ± 1.18 *	84.11 ± 1.35 *	221.2 ± 7.63 *	7.69 ± 0.27	14.82 ± 0.28	4.2 ± 0.15
'RB' II	52.11 ± 0.78	64.11 ± 0.65	103.91 ± 2.83	8.08 ± 0.22	15.40 ± 0.19	3.87 ± 0.17
'TO' I	60.59 ± 0.63 *	79.92 ± 0.65 *	191.39 ± 4.05 *	7.46 ± 0.19	15.51 ± 0.14	3.6 ± 0.18
'TO' II	54.29 ± 0.65	71.15 ± 0.79	157.56 ± 4.38	7.75 ± 0.19	15.30 ± 0.30	3.1 ± 0.28

Presented data are means ± standard error. Results marked with asterisk (*) represent statistically significant differences between the same cultivar from Groups I (70+ mm) and II (55–70 mm). No asterisk means there are no statistically significant differences between the same cultivar from Groups I and II, n.d.: not detected.

Color measurements were taken on both the sun-exposed and shaded sides of the fruit, and color parameters in the CIELAB color space (L*, a*, b*, C*, and h°) are shown in Table 2. No significant differences were found between size groups within cultivars,

confirming that size was the sole visible distinguishing factor. Visual inspection verified uniform color coverage between the two size groups.

3.2. Organic Acids and Sugars

Statistically significant differences were observed in the organic acid and sugar content of apple fruits across different size categories. Furthermore, significant variations were noted between the flesh and skin of apples within Groups I and II, as illustrated in Table 3. Differences were also detected between size groups I and II. Cultivars ‘BM’ and ‘CAR’ exhibited higher sucrose concentrations in the skin of apples from Group I. Conversely, cultivar ‘OP’ showed increased sucrose levels in the skin of smaller apples (Group II), while no significant differences were observed in sucrose content between the skins of ‘RB’ and ‘TO’ based on fruit size.

In terms of sucrose content in the flesh, cultivars ‘BM’, ‘RB’, and ‘CAR’ had higher concentrations in apples from Group I, whereas apples of ‘OP’ and ‘TO’ from Group II demonstrated greater sucrose content in the flesh. Glucose concentrations were notably higher in the skin of cultivar ‘OP’ in Group II, with no significant differences observed between the two size groups for other cultivars. In the flesh, glucose levels were elevated in apples from Group II for cultivars ‘BM’, ‘RB’, and ‘OP’, while no significant differences were found for ‘TO’ and ‘CAR’. Fructose content in the skin was higher for cultivar ‘BM’ in Group I, but no significant differences were found for ‘CAR’ and ‘TO’ in terms of skin fructose content between size groups. Cultivars ‘RB’ and ‘OP’ showed increased fructose levels in the skin of apples from Group II. Regarding flesh fructose, Group II apples of ‘BM’, ‘RB’, ‘OP’, and ‘TO’ exhibited higher fructose concentrations, with no significant difference in ‘CAR’ between size groups. The sorbitol content in the skin was higher in the larger apples of cultivar ‘BM’, while cultivar ‘OP’ had higher sorbitol concentrations in the skin of smaller apples. No significant differences in sorbitol content in the skin were found for other cultivars between Groups I and II. Sorbitol levels in the flesh were higher in Group II apples of ‘OP’, while no differences were detected for other cultivars.

Citric acid content was elevated in the skin of cultivar ‘CAR’ from Group II, but no significant differences were observed in citric acid levels in the skin for other cultivars based on fruit size. In the flesh, higher citric acid concentrations were found in Group II apples of ‘OP’ and ‘TO’. Malic acid content was higher in the skin of ‘OP’ from Group II, with no significant differences detected in malic acid levels in the skin for other cultivars. In the flesh, malic acid content was greater in Group I apples of cultivar ‘RB’, while ‘OP’ exhibited higher citric acid content in the flesh of Group II apples. No significant differences in malic acid content were observed between size groups for the remaining cultivars. Shikimic acid was the lowest measured organic acid across all studied cultivars, with the only significant difference being observed in cultivar ‘OP’, where higher shikimic acid concentrations were found in the flesh of apples from Group I.

Total sugar content, representing the sum of all identified sugars, was higher in the skin of cultivar ‘BM’ from the larger fruit group (Group I), while cultivar ‘OP’ exhibited higher total sugar content in the skin of smaller apples (Group II). For other cultivars (‘RB’, ‘TO’, and ‘CAR’), no significant differences in total sugar content in the skin were observed between size groups. In the flesh, total sugar content was higher for ‘BM’, ‘OP’, and ‘TO’ in Group II apples, while ‘CAR’ showed higher sugar content in the flesh of Group I apples. No significant differences in total sugar content based on fruit size were found for ‘RB’.

Table 2. Color measurements of sun-exposed side and shaded side of apple fruits of five different cultivars, from two size groups (I and II).

Cultivar	Sun-Exposed					Shaded Side				
	L*	a*	b*	C*	h°	L*	a*	b*	C*	h°
‘BM’ I	27.34 ± 0.42	22.43 ± 1.02	11.40 ± 0.58	25.15 ± 1.17	26.90 ± 0.19	48.30 ± 0.61	21.43 ± 1.03	28.91 ± 0.52	36.03 ± 0.42	54.36 ± 1.74
‘BM’ II	27.60 ± 0.35	23.47 ± 0.79	12.19 ± 0.48	26.46 ± 0.91	27.36 ± 0.25	47.99 ± 0.77	20.19 ± 1.48	27.63 ± 0.90	34.61 ± 0.31	54.69 ± 2.83
‘CAR’ I	34.12 ± 0.70	31.76 ± 1.07	19.05 ± 0.69	37.10 ± 1.08	31.02 ± 1.06	63.34 ± 0.80	0.87 ± 0.24	39.98 ± 0.52	39.99 ± 0.52	88.99 ± 0.44
‘CAR’ II	35.39 ± 1.35	31.89 ± 0.69	20.45 ± 1.67	38.11 ± 1.33	32.27 ± 2.03	61.06 ± 2.36	0.84 ± 0.14	41.08 ± 0.52	41.10 ± 0.52	89.04 ± 0.31
‘OP’ I	64.56 ± 0.43	5.68 ± 0.72	47.58 ± 0.55	47.95 ± 0.63	83.26 ± 0.76	n.d.	n.d.	n.d.	n.d.	n.d.
‘OP’ II	62.23 ± 0.51	3.91 ± 0.65	46.44 ± 0.65	46.64 ± 0.69	85.26 ± 0.74	n.d.	n.d.	n.d.	n.d.	n.d.
‘RB’ I	35.08 ± 1.01	27.98 ± 0.65	20.94 ± 1.42	35.15 ± 0.85	36.50 ± 2.10	55.51 ± 2.02	6.91 ± 0.78	42.14 ± 0.67	42.76 ± 0.62	80.64 ± 0.74
‘RB’ II	36.01 ± 0.80	30.36 ± 0.60	21.28 ± 0.67	37.10 ± 0.71	34.96 ± 0.87	56.07 ± 1.27	6.06 ± 0.67	42.37 ± 0.74	42.86 ± 0.72	81.84 ± 0.93
‘TO’ I	32.59 ± 0.60	31.81 ± 0.61	18.24 ± 0.54	36.69 ± 0.75	29.75 ± 0.50	58.40 ± 0.83	6.93 ± 0.64	45.88 ± 0.85	46.45 ± 0.90	81.86 ± 0.60
‘TO’ II	33.52 ± 0.56	32.29 ± 0.65	18.70 ± 0.63	37.08 ± 0.85	29.95 ± 0.47	58.19 ± 0.99	7.56 ± 0.56	45.05 ± 0.94	45.75 ± 0.97	80.49 ± 0.68

Presented data are means ± standard error. Results marked with asterisk (*) represent statistically significant differences between the same cultivar from Groups I (70+ mm) and II (55–70 mm). No asterisk means there are no statistically significant differences between the same cultivar from Groups I and II. Results presented as n.d. (no data) were not measured, as the color of the fruit was uniform (yellow-skinned apple cultivar) (*t*-test, *p* < 0.05).

Table 3. Individual organic acid and sugar content (g/kg FW) in skin and flesh of ‘BM’, ‘RB’, ‘CAR’, ‘OP’, and ‘TO’ of the group of sizes I and II.

Cultivar	Sugars					Organic Acids					
	Sucrose	Glucose	Fructose	Sorbitol	Total Sugars	Citric Acid	Malic Acid	Shikimic Acid	Total Organic Acids	Sugar/Acid Ratio	Sweetness
‘BM’ I skin	65.97 ± 5.24 *	16.89 ± 0.60	45.91 ± 1.44 *	9.08 ± 0.81 *	137.86 ± 7.36 *	6.46 ± 0.59	10.66 ± 0.97	0.07 ± 0.01	17.19 ± 1.52	8.08 ± 0.40 *	152.05 ± 7.46 *
‘BM’ II skin	39.48 ± 2.47	14.81 ± 1.38	36.28 ± 1.39	6.03 ± 0.53	96.59 ± 8.12	7.45 ± 0.40	9.06 ± 0.29	0.06 ± 0.01	16.57 ± 0.37	5.84 ± 0.55	108.01 ± 9.14
‘BM’ I flesh	41.58 ± 4.30 *	13.57 ± 1.78	43.25 ± 4.16	6.08 ± 1.03	104.48 ± 15.06	1.60 ± 0.06	9.25 ± 0.23	0.02 ± 0.00	10.87 ± 0.20	9.62 ± 1.41	119.67 ± 10.50
‘BM’ II flesh	35.53 ± 3.01	23.34 ± 1.33 *	61.32 ± 2.42 *	5.70 ± 0.84	125.88 ± 9.96 *	1.83 ± 0.24	8.66 ± 1.03	0.02 ± 0.00	10.51 ± 1.28	12.13 ± 0.74 *	147.86 ± 10.58 *
‘RB’ I skin	69.20 ± 4.22	19.47 ± 1.72	27.66 ± 1.21	6.67 ± 0.35	123.01 ± 4.79	7.32 ± 0.21	16.93 ± 1.06	0.06 ± 0.00	24.30 ± 1.91	5.09 ± 0.20	128.63 ± 4.17
‘RB’ II skin	63.05 ± 3.49	22.83 ± 1.65	34.75 ± 3.79 *	5.99 ± 0.58	126.63 ± 6.15	7.55 ± 1.58	14.90 ± 0.41	0.06 ± 0.00	22.51 ± 1.98	5.70 ± 0.53	135.30 ± 7.18
‘RB’ I flesh	54.78 ± 3.19 *	11.80 ± 0.43	39.19 ± 2.89	4.51 ± 0.68	110.28 ± 7.00	1.40 ± 0.09	9.95 ± 0.22 *	0.03 ± 0.00	11.38 ± 0.31	9.68 ± 0.39	124.67 ± 8.02
‘RB’ II flesh	45.60 ± 4.28	16.15 ± 1.53 *	47.88 ± 3.89 *	4.03 ± 0.32	113.66 ± 7.59	2.11 ± 0.45	7.88 ± 0.26	0.03 ± 0.00	10.02 ± 0.70	11.39 ± 0.78	131.55 ± 9.14
‘CAR’ I skin	60.22 ± 2.59 *	7.05 ± 1.06	42.98 ± 2.76	10.42 ± 1.12	120.68 ± 7.81	6.34 ± 0.15	9.44 ± 0.89	0.06 ± 0.00	15.84 ± 1.03	7.64 ± 0.39	135.20 ± 8.18
‘CAR’ II skin	51.64 ± 1.55	6.13 ± 0.51	46.49 ± 2.57	10.46 ± 1.45	114.72 ± 3.44	8.10 ± 1.24 *	8.15 ± 0.52	0.06 ± 0.01	16.31 ± 1.48	7.11 ± 0.42	131.20 ± 3.82
‘CAR’ I flesh	41.00 ± 1.86 *	5.18 ± 0.08	51.31 ± 0.66	6.67 ± 0.43	104.16 ± 2.27 *	1.51 ± 0.07	7.22 ± 0.62	0.03 ± 0.00	8.77 ± 0.38	12.01 ± 0.82	125.18 ± 2.70 *
‘CAR’ II flesh	33.72 ± 0.71	5.06 ± 0.30	49.72 ± 2.51	6.86 ± 0.96	95.36 ± 4.19	1.84 ± 0.26	6.13 ± 1.09	0.03 ± 0.00	8.01 ± 1.22	12.41 ± 1.62	115.53 ± 4.96
‘OP’ I skin	39.71 ± 3.28	6.43 ± 0.31	22.18 ± 2.78	2.67 ± 0.37	70.99 ± 7.73	5.09 ± 0.68	6.96 ± 0.79	0.04 ± 0.00	12.09 ± 1.27	5.83 ± 0.72	79.13 ± 4.86
‘OP’ II skin	58.20 ± 5.45 *	13.35 ± 1.47 *	30.94 ± 3.37 *	4.17 ± 0.17 *	106.65 ± 11.58 *	5.28 ± 0.60	9.42 ± 0.94 *	0.05 ± 0.01	14.75 ± 3.45	7.50 ± 0.69	116.70 ± 13.26 *

'OP' I flesh	32.44 ± 2.02	5.22 ± 0.39	28.47 ± 0.52	1.76 ± 0.12	67.88 ± 2.38	1.55 ± 0.23	5.87 ± 0.22	0.02 ± 0.00	7.44 ± 0.32	9.13 ± 0.20	79.93 ± 2.28
'OP' II flesh	57.52 ± 4.83 *	14.60 ± 1.48 *	50.96 ± 2.65 *	3.73 ± 0.60 *	126.82 ± 5.90 *	2.42 ± 0.35 *	10.26 ± 1.02 *	0.04 ± 0.00 *	12.71 ± 1.21 *	10.09 ± 0.68	146.78 ± 6.06 *
'TO' I skin	75.66 ± 6.97	8.63 ± 0.58	26.50 ± 2.88	4.16 ± 0.53	114.94 ± 9.82	4.10 ± 0.50	8.62 ± 0.39	0.04 ± 0.00	12.74 ± 0.76	8.99 ± 0.24	123.95 ± 11.07
'TO' II skin	70.48 ± 1.00	8.21 ± 0.46	25.73 ± 0.94	3.97 ± 0.43	108.39 ± 0.95	3.50 ± 0.18	7.50 ± 0.10	0.04 ± 0.00	11.04 ± 0.07	9.82 ± 0.14	117.22 ± 1.06
'TO' I flesh	50.02 ± 0.14	7.83 ± 1.61	28.87 ± 0.93	2.81 ± 0.13	89.52 ± 1.84	1.41 ± 0.18	6.68 ± 0.75	0.02 ± 0.00	8.12 ± 0.62	11.17 ± 0.91	100.59 ± 1.81
'TO' II flesh	67.05 ± 3.75 *	8.77 ± 0.94	37.21 ± 3.58 *	3.76 ± 0.65	116.80 ± 8.20 *	2.37 ± 0.24 *	7.65 ± 0.74	0.03 ± 0.01	10.05 ± 0.98	11.68 ± 0.33	131.32 ± 9.55 *

Presented data are means ± standard error. Results marked with asterisk (*) represent statistically significant differences ($p < 0.05$) between the same cultivar from Groups I (70+ mm) and II (55–70 mm). No asterisk means there are no statistically significant differences between the same cultivar from Groups I and II. Statistically significant differences were detected between the size groups I and II (t -test, $p < 0.05$).

Total organic acid content was greater only in the flesh of smaller apples for cultivar 'OP', with no significant differences observed between size groups for other cultivars, either in the skin or flesh.

The sugar/acid ratio was significantly higher in the skin of 'BM' apples from Group I, with no differences observed for other cultivars between the two size groups. The sweetness index varied between Group I and II apples, depending on the cultivar.

3.3. Identification of Phenolic Compounds

Based on the literature and mass spectra, 46 phenolic compounds were identified in the skin and flesh of the five cultivars: 19 hydroxycinnamic acids, 1 hydroxybenzoic acid, 2 dihydrochalcones, 3 anthocyanins, 6 flavonols, and 15 flavanols (Table 4). All the identified compounds were previously identified in our previous study, where the identification is explained in detail [20].

3.4. Phenolic Content

The quantification of individual phenolic compounds and the total phenolic content in the skin (Table 5) and flesh (Table 6) are presented below.

Total hydroxycinnamic acid concentrations were significantly higher only in the skin of Group I apples of cultivar 'BM'. No significant differences were observed between size groups for the other cultivars. In contrast, total hydroxybenzoic acid levels were significantly greater in Group I apples of 'BM' and in Group II apples of cultivars 'CAR' and 'TO'. No differences based on size were detected for the remaining cultivars.

Total dihydrochalcone content was higher in the skin of Group II apples of cultivars 'OP' and 'CAR', while no statistically significant differences were found for other cultivars. The total flavonol content was higher in the skin of Group II apples from cultivars 'RB', 'TO', and 'CAR', while 'BM' exhibited higher flavonol content in Group I apples. No size-dependent differences were observed for 'OP'.

No statistically significant differences were observed in total flavanol content based on apple size. Total anthocyanin content was higher in the skin of 'BM' and 'TO' from Group I, whereas 'RB' and 'CAR' had higher anthocyanin levels in smaller fruits. No anthocyanins were detected in the skin of 'OP', as expected, given its yellow/green skin phenotype. Total anthocyanin and phenolic compounds (TAPC) were higher in the skin of 'BM' Group I, 'TO' Group I, and 'CAR' Group II apples. No significant size-dependent differences were found for other cultivars.

Total flavonol content was the only phenolic compound group that was higher in the flesh of larger apples from the red-fleshed cultivar 'BM'. No significant differences in the total content of other phenolic compound groups were observed. TAPC values were similar between size groups I and II for most cultivars. However, the TAPC of 'RB' was higher in Group II apples, primarily due to increased levels of total flavanols, dihydrochalcones, and hydroxycinnamic acids. Similarly, 'CAR' had higher TAPC in smaller apples, driven by an increase in dihydrochalcone content. Cultivars 'OP' and 'TO' exhibited higher TAPC in Group II apples, as their total hydroxycinnamic acid and flavanol contents were higher in smaller fruits.

Interestingly, the total flavanol content in red-fleshed 'BM' was approximately 10 times lower than that in other white-fleshed cultivars studied.

Table 4. Tentative identification of 46 phenolic compounds of *Malus domestica* cultivars: ‘BM’, ‘RB’, ‘CAR’, ‘OP’, and ‘TO’.

Phenolic Compounds	Rt (min)	[M – H] [–] (m/z)	[M + H] ⁺ (m/z)	MS ² (m/z)	‘BM’		‘RB’		‘TO’		‘CAR’		‘OP’	
					Peel	Flesh	Peel	Flesh	Peel	Flesh	Peel	Flesh	Peel	Flesh
Cyanidin-3- <i>O</i> -galactoside	9.41		449	287			X		X		X			
Caffeic acid hexoside 1	10.04	341		179,161,135									X	
<i>p</i> -Coumaric acid hexoside derivative 1	10.1	371		325,163,119	X		X		X		X		X	
Cyanidin-3- <i>O</i> -arabinoside	10.61		419	287	X									
Dicafeic acid derivative 1	11.0	457		277,189,179	X		X				X	X		
Procyanidin dimer 1	11.38	577		451,425,407,289,287			X	X	X	X	X	X	X	X
Caffeic acid derivative 1	11.6	311		267,249,205,153					X				X	X
Ferulic acid hexoside derivative	11.62	401		355	X	X		X				X		
<i>p</i> -Coumaric acid hexoside derivative 2	12.07	371		325,163,119	X				X				X	
Cyanidin-3- <i>O</i> -xyloside	12.37		419	287	X		X		X		X			
Caffeic acid hexoside 2	12.4	341		313,295,281,251,221	X									
Neochlorogenic acid (3-cafeoylquinic acid)	12.7	353		191,179,173,135			X						X	
(+)Catehin	13.10	289		245,205,203,179							X		X	X
<i>p</i> -Coumaric acid hexoside 1	13.25	325		265,235,163	X								X	
Procyanidin trimer 1	13.35	865		739,713,695,587,577,575,407			X		X				X	X
Chlorogenic acid (5-cafeoylquinic acid)	13.6	353		191,179,135	X	X	X	X	X	X	X	X	X	X
Caffeoylferuoylquinic acid	13.77	563		517,289	X	X	X		X			X	X	X
Protocatechuic acid derivative	14.03	481		345,327	X	X	X		X		X			
Cryptochlorogenic acid (4-cafeoylquinic acid)	14.25	353		191,179				X		X				
Ferulic acid hexoside	14.4	355		295,265,235,193				X			X	X	X	
Procyanidin dimer 2	14.76	577		425,407,451	X	X	X	X	X	X	X	X	X	X
Procyanidin tetramer 1	14.95	1153		864,1000,863, 575			X		X					X
<i>p</i> -Coumaric acid hexoside 2	15.1	325		265,235,163										X
Procyanidin tetramer 2	15.35	1153		864,1000,863,575			X							
(-)Epicatehin	15.96	289		245,231,205,179	X	X	X	X	X	X	X	X	X	X
4- <i>O</i> - <i>p</i> -coumaroylquinic acid	16.2	337		191,173,163									X	X
5- <i>O</i> - <i>p</i> -coumaroylquinic acid	16.95	337		191,179,173,163		X	X	X				X	X	X
Caffeic acid derivative	17.0	335		179,135	X				X					
Procyanidin trimer 2	17.14	865		739,695,577	X	X	X	X	X	X	X	X	X	X
Procyanidin dimer 3	17.7	577		451,425,407,289,287			X		X				X	X

Procyanidin tetramer 3	17.8	1153	864,1000,863,575	X	X	X		X	X		X	X
Procyanidin trimer 3	18.55	865	739,713,695,587,577,575,407			X		X				
Quercetin-3- <i>O</i> -rutinoside	20.35	609	343,301,300,179	X		X		X		X		
(Epi)catechin derivative 1	20.36	583	289,271,167			X	X			X		
Procyanidin dimer 4	20.57	577	451,425,407,289,287,245			X	X	X	X	X	X	X
Dihydrodicaffeic acid derivative	20.63	405	225,181			X	X					
Quercetin-3- <i>O</i> -galactoside	21.14	463	301,300,179	X	X	X		X	X	X		X
Quercetin-3- <i>O</i> -glucoside	21.2	463	301,300,179	X	X	X		X	X	X		X
Dihydroprocyanidin dimer	21.6	579	289,245,203	X	X		X	X	X	X	X	X
Quercetin-3- <i>O</i> -arabinofuranoside	22.06	433	301,300,179	X		X		X	X	X		X
Phloretin-2- <i>O</i> -xyloside	22.11	567	273,167	X	X	X	X	X	X	X	X	X
Dicaffeic acid derivative 3	22.3	429	249,205,179,135	X	X	X	X	X	X	X	X	X
Quercetin-3- <i>O</i> -arabinopyranoside	22.77	433	301,300,179	X	X	X	X	X		X		
Quercetin-3- <i>O</i> -rhamnoside	23.0	447	301,300,179	X	X	X	X	X	X		X	X
(Epi)catechin derivative 3	23.55	477	331,330,316,289			X						
Phloridzin	23.77	481	435	X	X	X	X	X	X	X	X	X

Rt: retention time; [M – H][–]: pseudomolecular ion identified in a negative ion mode; [M + H]⁺: pseudomolecular ion identified in a positive ion mode; X: presence of the identified compound.

Table 5. Individual and total analyzed phenolic compounds (TAPC) in the skin of five apple (*Malus domestica*) cultivars: ‘BM’, ‘RB’, ‘CAR’, ‘OP’, and ‘TO’ (mean ± SE, in mg/kg FW).

Compounds	Individual Phenolic Compounds in the Skin (mg/kg FW)									
	‘BM’ I	‘BM’ II	‘RB’ I	‘RB’ II	‘CAR’ I	‘CAR’ II	‘OP’ I	‘OP’ II	‘TO’ I	‘TO’ II
Hydroxycinnamic acids										
<i>p</i> -coumaric acid hexoside derivative 1	3.67 ± 0.33 *	2.22 ± 0.28	1.73 ± 0.23	2.50 ± 0.36	2.56 ± 0.16	2.86 ± 0.45	nd	nd	5.91 ± 0.62	5.99 ± 0.42
<i>p</i> -coumaric acid hexoside derivative 2	5.30 ± 0.58	3.96 ± 0.29	nd	nd	nd	nd	1.56 ± 0.20	1.57 ± 0.17	4.86 ± 0.21	4.37 ± 0.20
<i>p</i> -coumaroyl hexoside derivative	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
<i>p</i> -coumaric acid hexoside	4.40 ± 0.32 *	2.46 ± 0.24	nd	nd	nd	nd	1.52 ± 0.18	1.46 ± 0.35	nd	nd
<i>p</i> -coumaric acid hexoside 1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
<i>p</i> -coumaric acid hexoside 2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Caffeic acid derivative	2.34 ± 0.34 *	1.22 ± 0.21	nd	nd	nd	nd	nd	nd	2.59 ± 0.23	2.42 ± 0.33
Caffeic acid derivative 2	nd	nd	nd	nd	nd	nd	1.46 ± 0.23 b	2.00 ± 0.35 a	nd	nd
Dicaffeic acid derivative	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Dicaffeic acid derivative 1	2.72 ± 0.42 *	1.47 ± 0.20	2.27 ± 0.34	1.54 ± 0.25	2.35 ± 0.27	4.31 ± 0.37 *	nd	nd	nd	nd
Dicaffeic acid derivative 2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Dicaffeic acid derivative 3	1.36 ± 0.08	1.69 ± 0.25	1.91 ± 0.24	2.45 ± 0.33	2.42 ± 0.27	2.91 ± 0.35	3.45 ± 0.50	5.20 ± 0.90	3.84 ± 0.16	3.27 ± 0.21
Caffeic acid hexoside 1	nd	nd	nd	nd	nd	nd	2.27 ± 0.32	2.82 ± 0.63	nd	nd

Caffeic acid hexoside 2	8.01 ± 0.76 *	4.69 ± 0.81	nd	nd	nd	nd	nd	nd	nd	nd
Dihydrocaffeic acid derivative	nd	nd	1.24 ± 0.29	1.38 ± 0.26	nd	nd	nd	nd	nd	nd
Dihydrodicaffeic acid derivative	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4- <i>O-p</i> -coumaroylquinic acid	nd	nd	nd	nd	nd	nd	5.98 ± 0.70	6.11 ± 0.64	nd	nd
5- <i>O-p</i> -coumaroylquinic acid	nd	nd	4.42 ± 0.75	4.80 ± 0.23	nd	nd	5.81 ± 0.87	6.65 ± 0.66	nd	nd
Chlorogenic acid (5-caffeoylquinic acid)	15.62 ± 1.29 *	10.39 ± 0.70	43.09 ± 1.72	59.02 ± 1.67 *	11.21 ± 1.06	13.47 ± 0.64	7.89 ± 1.44	9.02 ± 2.47	46.76 ± 2.48	58.90 ± 5.41
Neochlorogenic acid	nd	nd	12.08 ± 1.17	12.77 ± 1.37	nd	nd	5.10 ± 1.34	6.97 ± 1.88	nd	nd
Caffeoylferoylquinic acid	5.97 ± 0.42	5.54 ± 0.45	7.55 ± 0.64	9.27 ± 1.73	nd	nd	6.77 ± 1.42	6.54 ± 1.23	nd	nd
Feruloylquinnic acid gallate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ferulic acid hexoside	nd	nd	nd	nd	2.88 ± 0.35	3.78 ± 0.49	3.52 ± 0.46	3.58 ± 0.54	nd	nd
Ferulic acid hexoside derivative	1.38 ± 0.14	1.18 ± 0.13	nd	nd	nd	nd	nd	nd	nd	nd
Cryptochlorogenic acid (4-caffeoylquinic acid)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Hydroxybenzoic acids										
Protocatechuic acid derivative	89.58 ± 8.49 *	51.58 ± 4.95	20.83 ± 1.49	27.05 ± 2.42	46.77 ± 4.26	61.63 ± 4.46 *	nd	nd	38.75 ± 2.50 *	29.67 ± 1.73
Dihydrochalcones										
Phloridzin	172.32 ± 9.95	156.45 ± 9.04	268.70 ± 16.61 *	211.56 ± 13.88	98.32 ± 8.56	165.72 ± 13.32 *	95.93 ± 4.86	156.83 ± 11.42 *	61.03 ± 3.87	74.35 ± 4.25
Phloretin-2- <i>O</i> -xyloside	116.63 ± 12.61 *	99.41 ± 4.68	136.45 ± 8.59	146.02 ± 8.67	221.04 ± 18.66	345.91 ± 29.42 *	24.91 ± 2.62	29.80 ± 3.28	41.07 ± 2.45 *	33.22 ± 2.28
Flavonols										
Quercetin-3- <i>O</i> -arabinofuranoside	13.03 ± 1.24	12.07 ± 1.42	26.82 ± 1.88	28.40 ± 2.47	21.11 ± 1.95	35.14 ± 2.84 *	30.88 ± 2.59	37.42 ± 3.12	20.01 ± 1.33	18.64 ± 0.83
Quercetin-3- <i>O</i> -arabinopyranoside	37.73 ± 3.79 *	26.02 ± 2.07	25.21 ± 2.59	35.13 ± 2.84 *	50.52 ± 4.14	109.42 ± 9.72 *	28.06 ± 2.46	23.39 ± 2.68	59.11 ± 5.84	57.74 ± 4.20
Quercetin-3- <i>O</i> -galactoside	104.38 ± 9.37 *	62.83 ± 5.71	66.66 ± 4.53	131.38 ± 11.53 *	146.39 ± 11.93	360.30 ± 23.19 *	30.24 ± 3.10	30.00 ± 3.02	189.35 ± 11.23 *	136.65 ± 13.02
Quercetin-3- <i>O</i> -glucoside	12.88 ± 2.20	9.46 ± 1.23	10.94 ± 0.69	17.23 ± 1.11 *	54.07 ± 4.66	122.28 ± 11.67 *	9.92 ± 1.03	10.80 ± 1.22	30.36 ± 2.37	25.39 ± 2.35
Quercetin-3- <i>O</i> -rhamnoside	24.63 ± 2.75 *	15.80 ± 1.98	25.45 ± 2.95	34.04 ± 2.56 *	nd	nd	32.60 ± 3.18	26.68 ± 3.51	312.03 ± 15.74 *	280.39 ± 8.66
Quercetin-3- <i>O</i> -rutinoside	19.22 ± 2.16	14.92 ± 1.82	11.56 ± 1.19	24.68 ± 2.27 *	15.15 ± 2.14	24.37 ± 2.25 *	nd	nd	35.67 ± 2.29 *	21.79 ± 1.37
Quercetin-3- <i>O</i> -xyloside	nd	nd	nd	nd	27.41 ± 2.47	48.76 ± 4.67 *	nd	nd	nd	nd
Flavanols										
(-)epicatehin	51.87 ± 4.20 *	40.52 ± 2.25	153.17 ± 10.47	166.48 ± 10.53	207.78 ± 9.25	251.49 ± 12.13 *	121.74 ± 11.08	134.17 ± 11.96	nd	nd
Epicatechin derivative 1	nd	nd	42.20 ± 2.35	59.08 ± 5.04 *	111.78 ± 9.75	188.43 ± 15.73 *	nd	nd	nd	nd
Epicatechin derivative 2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Epicatechin derivative 3	nd	nd	7.12 ± 1.70	8.09 ± 1.04	nd	nd	nd	nd	nd	nd
(+)catechin	nd	nd	nd	nd	80.84 ± 5.24	121.51 ± 11.26 *	41.79 ± 2.72	59.15 ± 3.74 *	284.21 ± 18.99	253.17 ± 10.04
Flavanol monomer	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Procyanidin dimer 1	nd	nd	46.95 ± 3.21	58.79 ± 5.99	41.01 ± 4.57	68.52 ± 5.91 *	58.75 ± 5.62	52.23 ± 5.23	33.82 ± 3.61	34.38 ± 3.31
Procyanidin dimer 2	65.46 ± 6.22	62.38 ± 3.93	238.32 ± 27.06	248.56 ± 18.59	271.15 ± 15.59	345.55 ± 19.82 *	113.66 ± 7.87	120.09 ± 8.35	296.44 ± 23.98	271.20 ± 22.03
Procyanidin dimer 3	nd	nd	15.64 ± 2.23	28.10 ± 2.26 *	nd	nd	6.30 ± 0.93	10.35 ± 1.09 *	nd	nd
Procyanidin dimer 4	nd	nd	53.91 ± 5.14 *	39.42 ± 4.13	48.51 ± 4.13	63.61 ± 5.47 *	29.34 ± 2.92	35.91 ± 3.13	30.82 ± 3.09	29.92 ± 2.99
Procyanidin trimer 1	nd	nd	90.31 ± 4.78	133.30 ± 11.19 *	nd	nd	36.96 ± 3.51	35.93 ± 3.03	nd	nd
Procyanidin trimer 2	32.74 ± 2.99	33.17 ± 2.52	163.80 ± 13.99	186.56 ± 14.64	198.92 ± 14.48	250.94 ± 24.64 *	183.44 ± 16.79	191.31 ± 18.56	130.13 ± 13.07	128.31 ± 12.01
Procyanidin trimer 3	nd	nd	25.05 ± 2.63	36.23 ± 2.20 *	nd	nd	16.02 ± 1.94	17.06 ± 2.10	nd	nd
Procyanidin tetramer 1	nd	nd	32.42 ± 3.39	38.46 ± 3.85	nd	nd	nd	nd	nd	nd
Procyanidin tetramer 2	nd	nd	38.00 ± 2.09	42.27 ± 3.92	nd	nd	nd	nd	nd	nd
Procyanidin tetramer 3	13.56 ± 1.47	18.42 ± 1.54	58.10 ± 4.44	67.68 ± 6.32	59.13 ± 5.20	93.13 ± 6.89 *	36.68 ± 4.69	33.40 ± 3.41	nd	nd

Dihydroprocyanidin dimer	44.75 ± 4.54	44.07 ± 3.84	nd	nd	86.93 ± 6.14	98.18 ± 8.13	37.11 ± 3.74	43.46 ± 4.61	179.44 ± 11.39	167.04 ± 14.28
Anthocyanins										
Cyanidin-3-galactoside	379.57 ± 28.15 *	139.69 ± 12.47	59.22 ± 4.70	80.21 ± 5.59 *	73.62 ± 6.65	125.39 ± 11.36 *	nd	nd	182.54 ± 13.56 *	128.53 ± 10.91
Cyanidin-3-arabinoside	26.83 ± 2.29 *	12.16 ± 0.64	4.51 ± 0.89	6.01 ± 0.72	4.98 ± 0.61	10.46 ± 1.97 *	nd	nd	11.08 ± 0.42 *	7.45 ± 0.76
Cyanidin-3-xyloside	51.61 ± 3.80 *	24.42 ± 2.76	4.58 ± 0.46	4.64 ± 0.44	3.76 ± 0.39	7.86 ± 1.04 *	nd	nd	11.08 ± 0.70 *	7.88 ± 0.89
Total hydroxycinnamic acids	50.76 ± 4.45 *	34.81 ± 2.04	74.29 ± 4.67	93.73 ± 13.81	21.42 ± 2.52	27.34 ± 1.26	45.34 ± 6.61	51.93 ± 9.98	21.42 ± 2.52	27.34 ± 1.26
Total hydroxybenzoic acids	89.58 ± 11.49 *	51.58 ± 4.96	20.83 ± 1.49	27.05 ± 3.42	46.77 ± 4.26	61.63 ± 4.46 *	nd	nd	46.77 ± 4.26	61.63 ± 4.46 *
Total dihydrochalcones	288.95 ± 20.31	255.86 ± 13.42	405.15 ± 24.68	357.57 ± 22.07	319.35 ± 27.04	511.63 ± 42.38 *	120.84 ± 7.06	186.64 ± 15.43 *	102.10 ± 6.07	107.57 ± 6.23
Total flavonols	211.87 ± 32.21 *	141.10 ± 20.39	166.64 ± 16.04	270.87 ± 38.76 *	314.65 ± 25.57	700.27 ± 94.68 *	131.70 ± 14.00	128.29 ± 14.57	314.65 ± 25.57	700.27 ± 94.68 *
Total flavanols	208.37 ± 22.43	198.56 ± 13.45	950.50 ± 76.06	1127.53 ± 146.22	1106.03 ± 118.66	1481.37 ± 124.97	742.22 ± 60.94	733.08 ± 76.70	1106.03 ± 118.66	1481.37 ± 124.97
Total anthocyanins	458.01 ± 44.09 *	176.27 ± 19.38	68.32 ± 3.26	90.86 ± 11.60 *	82.37 ± 8.61	143.71 ± 17.19 *	nd	nd	204.71 ± 24.34 *	143.87 ± 14.49
TAPC	1307.54 ± 132.11 *	858.17 ± 46.39	1685.72 ± 102.15	1967.62 ± 221.52	1890.59 ± 178.80	2925.95 ± 210.32 *	979.67 ± 103.61	1099.93 ± 137.59	2010.93 ± 140.63 *	1780.69 ± 78.97

Presented data are means ± standard error. Results marked with asterisk (*) represent statistically significant differences ($p < 0.05$) between the same cultivar from Groups I (70+ mm) and II (55–70 mm). No asterisk means there are no statistically significant differences between the same cultivar from Groups I and II. nd, not detected. The comparison is made based on size group I (70+ mm) and size group II (55–70 mm). Letters ‘nd’ stand for no data (t -test, $p < 0.05$).

Table 6. Individual and total analyzed phenolic compounds (TAPC) in the flesh of five apple (*Malus domestica*) cultivars: ‘BM’, ‘RB’, ‘CAR’, ‘OP’, and ‘TO’ (mean ± SE, in mg/kg FW).

Compounds	Individual Phenolic Compounds in the Flesh (mg/kg FW)									
	‘BM’ I	‘BM’ II	‘RB’ I	‘RB’ II	‘CAR’ I	‘CAR’ II	‘OP’ I	‘OP’ II	‘TO’ I	‘TO’ II
Hydroxycinnamic acids										
<i>p</i> -coumaric acid hexoside 1	nd	nd	nd	nd	nd	nd	0.31 ± 0.02	0.41 ± 0.05	nd	nd
Caffeic acid derivative 2	nd	nd	nd	nd	nd	nd	0.23 ± 0.02	0.32 ± 0.03	0.25 ± 0.03	0.40 ± 0.04 *
Dicafeic acid derivative 1	nd	nd	nd	nd	0.87 ± 0.07	1.26 ± 0.05 *	nd	nd	nd	nd
Dicafeic acid derivative 2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Dicafeic acid derivative 3	0.37 ± 0.08	0.40 ± 0.02	nd	nd	0.46 ± 0.02	0.72 ± 0.02 *	0.41 ± 0.04	0.58 ± 0.05	1.15 ± 0.12	1.63 ± 0.16 *
Dicafeic acid derivative 4	nd	nd	0.57 ± 0.08	1.05 ± 0.17 *	nd	nd	nd	nd	nd	nd
Dihydrocaffeic acid derivative	nd	nd	0.20 ± 0.02	0.30 ± 0.05	nd	nd	nd	nd	nd	nd
4- <i>O-p</i> -coumaroylquinic acid	nd	nd	nd	nd	nd	nd	0.44 ± 0.03	0.60 ± 0.05	nd	nd
5- <i>O-p</i> -coumaroylquinic acid	2.03 ± 0.30	1.59 ± 0.14	4.39 ± 0.52	7.28 ± 1.59 *	7.17 ± 0.75	5.23 ± 0.67	5.14 ± 0.56	7.01 ± 1.11	nd	nd
Chlorogenic acid (5-caffeoylquinic acid)	11.13 ± 1.51	10.38 ± 0.95	75.02 ± 6.13	111.39 ± 8.16 *	38.36 ± 3.45	41.51 ± 2.44	10.62 ± 0.64	13.81 ± 0.71	36.33 ± 1.32	43.12 ± 3.62
Caffeoylferuoylquinic acid	0.56 ± 0.09	0.53 ± 0.09	nd	nd	4.53 ± 0.26	4.20 ± 0.29	0.87 ± 0.15	1.75 ± 0.30 *	1.54 ± 0.18	1.68 ± 0.22
Feruloylquinnic acid gallate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ferulic acid hexoside	nd	nd	1.32 ± 0.33	1.20 ± 0.19	0.97 ± 0.04	1.00 ± 0.04	nd	nd	nd	nd
Ferulic acid hexoside derivative	0.22 ± 0.04	0.21 ± 0.02	0.27 ± 0.04	0.45 ± 0.06	0.46 ± 0.03	0.61 ± 0.03 *	nd	nd	nd	nd
Cryptochlorogenic acid (4-caffeoylquinic acid)	nd	nd	3.71 ± 0.47	4.95 ± 0.68	nd	nd	nd	nd	0.34 ± 0.04	0.57 ± 0.15
Hydroxybenzoic acids										
Protocatechuic acid derivative	3.80 ± 0.02	3.36 ± 0.44	nd	nd	nd	nd	nd	nd	nd	nd
Dihydrochalcones										
Phloridzin	3.95 ± 0.54	4.40 ± 0.55	13.15 ± 1.45	23.58 ± 2.52 *	4.72 ± 0.44	5.37 ± 0.29	7.63 ± 1.10	9.52 ± 0.84	3.92 ± 0.57	4.06 ± 0.36
Phloretin-2- <i>O</i> -xyloside	6.37 ± 1.10	7.36 ± 0.65	17.80 ± 1.98	29.40 ± 1.83 *	31.69 ± 3.30	41.66 ± 3.20 *	2.96 ± 0.37	3.23 ± 0.33	4.27 ± 0.35	4.73 ± 0.52

Flavonols										
Quercetin-3- <i>O</i> -arabinofuranoside	nd	nd	nd	nd	nd	nd	0.55 ± 0.04 a	0.69 ± 0.06 a	nd	nd
Quercetin-3- <i>O</i> -arabinopyranoside	0.63 ± 0.08	0.37 ± 0.02	0.55 ± 0.03	0.76 ± 0.10	nd	nd	nd	nd	nd	nd
Quercetin-3- <i>O</i> -galactoside	0.99 ± 0.14 *	0.50 ± 0.04	nd	nd	nd	nd	0.16 ± 0.01	0.23 ± 0.03	0.34 ± 0.02	0.31 ± 0.05
Quercetin-3- <i>O</i> -glucoside	0.59 ± 0.07	0.36 ± 0.02	nd	nd	nd	nd	0.25 ± 0.07	0.23 ± 0.01	0.16 ± 0.01	0.13 ± 0.01
Quercetin-3- <i>O</i> -rhamnoside	1.31 ± 0.19	1.02 ± 0.07	0.66 ± 0.07	0.69 ± 0.10	0.97 ± 0.03	0.95 ± 0.06	0.82 ± 0.08	0.89 ± 0.06	0.92 ± 0.02	1.00 ± 0.11
Flavanols										
(-)epicatehin	1.60 ± 0.51	2.14 ± 0.28	39.99 ± 3.54	50.12 ± 2.32 *	46.72 ± 2.88	45.57 ± 1.74	29.68 ± 1.45	47.68 ± 3.79 *	32.48 ± 1.17	44.42 ± 3.93 *
Epicatechin derivative 1	nd	nd	3.88 ± 0.51	5.21 ± 0.40	nd	nd	nd	nd	nd	nd
(+)catechin	nd	nd	nd	nd	nd	nd	7.87 ± 0.59	8.11 ± 0.21	nd	nd
Procyanidin dimer 1	nd	nd	16.75 ± 1.33	32.90 ± 3.34 *	13.28 ± 1.59	16.43 ± 0.69	11.07 ± 0.83	23.34 ± 2.95 *	8.72 ± 0.83	14.26 ± 1.51 *
Procyanidin dimer 2	5.96 ± 1.28	5.79 ± 0.83	84.44 ± 6.12	126.17 ± 12.30 *	114.77 ± 9.62	126.63 ± 3.05	66.43 ± 6.84	98.89 ± 8.78 *	89.89 ± 3.02	132.43 ± 11.39 *
Procyanidin dimer 3	nd	nd	nd	nd	nd	nd	2.20 ± 0.23	3.90 ± 0.28	2.54 ± 0.21	3.00 ± 0.40
Procyanidin dimer 4	nd	nd	6.24 ± 0.69	6.79 ± 0.55	7.18 ± 1.01	8.03 ± 0.65	4.16 ± 0.33	3.79 ± 0.28	6.03 ± 0.79	5.60 ± 0.79
Procyanidin trimer 1	nd	nd	nd	nd	nd	nd	11.34 ± 0.74	11.52 ± 0.80	6.84 ± 0.42	6.51 ± 0.38
Procyanidin trimer 2	8.92 ± 1.62	8.55 ± 1.56	54.19 ± 3.82	70.05 ± 4.17 *	10.28 ± 1.84	19.97 ± 2.53 *	21.66 ± 1.83	18.25 ± 2.50	30.64 ± 1.39	36.20 ± 3.51
Procyanidin trimer 3	nd	nd	nd	nd	nd	nd	nd	nd	1.43 ± 0.16	2.86 ± 0.66 *
Procyanidin tetramer 1	nd	nd	nd	nd	nd	nd	2.84 ± 0.32	2.54 ± 0.19	3.91 ± 0.34	5.88 ± 0.61
Procyanidin tetramer 3	0.92 ± 0.08	0.83 ± 0.17	nd	nd	nd	nd	4.55 ± 0.71	3.15 ± 0.47	4.89 ± 0.54	7.42 ± 0.86
Dihydroprocyanidin dimer	7.60 ± 1.05	6.95 ± 0.35	12.08 ± 1.07	25.16 ± 2.31 *	22.87 ± 1.52	21.40 ± 1.66	7.85 ± 0.72	10.30 ± 1.22	8.32 ± 0.62	7.62 ± 0.62
Anthocyanins										
Cyanidin-3-galactoside	37.92 ± 2.32	35.93 ± 2.31	nd	nd	nd	nd	nd	nd	nd	nd
Cyanidin-3-arabinoside	2.07 ± 0.38	2.42 ± 0.39	nd	nd	nd	nd	nd	nd	nd	nd
Cyanidin-3-xyloside	7.90 ± 1.08	6.63 ± 1.47	nd	nd	nd	nd	nd	nd	nd	nd
Total hydroxycinnamic acids	14.32 ± 1.30	13.11 ± 1.17	85.49 ± 8.17	126.63 ± 14.11 *	52.83 ± 3.10	54.54 ± 1.84	18.03 ± 1.34	24.47 ± 2.11 *	39.61 ± 1.54	47.40 ± 3.73 *
Total hydroxybenzoic acids	3.80 ± 0.30	3.36 ± 0.44	nd	nd	nd	nd	nd	nd	nd	nd
Total dihydrochalcones	10.32 ± 1.58	11.76 ± 1.18	30.95 ± 3.37	52.98 ± 4.20 *	36.41 ± 3.60	47.03 ± 3.18 *	10.60 ± 1.43	12.74 ± 1.15	8.19 ± 0.92	8.79 ± 0.88
Total flavonols	3.52 ± 0.49 *	2.24 ± 0.14	1.21 ± 0.09	1.46 ± 0.21	0.97 ± 0.03	0.95 ± 0.07	1.78 ± 0.19	2.04 ± 0.16	1.41 ± 0.03	1.45 ± 0.17
Total flavanols	24.99 ± 4.89	24.26 ± 2.84	217.56 ± 26.99	316.39 ± 33.39 *	215.10 ± 18.15	238.04 ± 5.14	169.65 ± 8.11	231.50 ± 14.07 *	195.69 ± 5.67	266.20 ± 28.45 *
Total anthocyanins	46.89 ± 6.68	44.12 ± 2.80	nd	nd	nd	nd	nd	nd	nd	nd
TAPC	103.83 ± 10.96	98.85 ± 7.51	335.21 ± 44.11	497.46 ± 55.88 *	305.30 ± 20.27	340.56 ± 8.63 *	200.05 ± 10.06	270.75 ± 17.37 *	244.90 ± 7.34	323.84 ± 32.21 *

Presented data are means ± standard error. Results marked with asterisk (*) represent statistically significant differences ($p < 0.05$) between the same cultivar from Groups I (70+ mm) and II (55–70 mm). No asterisk means there are no statistically significant differences between the same cultivar from Groups I and II. nd: not detected. The comparison is made based on size group I (70+ mm) and size group II (55–70 mm). Letters 'nd' stand for no data (t -test, $p < 0.05$).

3.5. Aroma Profile

A total of six groups of volatile organic compounds (VOCs) were identified, including esters (15 compounds), alcohols (4), aldehydes (5), ketones (3), acids (1), and terpenes (2). The majority of VOCs in both the skin (Table 7) and flesh (Table 8) of apple fruits from Group I and Group II were aldehydes, while acids were the least represented group. VOC content varied between Group I and Group II apples, depending on the cultivar, in both the skin and flesh.

Total ester content was lowest in the skin of cultivar 'RB', particularly in the larger apples from Group I (567.79 µg/kg FW), while cultivar 'P' exhibited the highest total ester content, especially in the skin of larger apples (Group I). Major esters included butyl acetate, hexyl acetate, hexyl 2-methylbutanoate, and hexyl butyrate. The red-fleshed cultivar 'BM' had a high total alcohol content in both smaller and larger apples, primarily due to elevated butyl alcohol levels. Among the aldehydes, n-hexanal and 2-E-hexenal were the most abundant VOCs, contributing significantly to the total aldehyde content. While 'BM' and 'RB' had higher total aldehyde content in the skin of Group II apples, no statistically significant differences were observed for other cultivars across the different fruit sizes. 2-Propanone was the major ketone, but no consistent trend in its distribution between Group I and Group II apples was found, as it varied with cultivar. Acetic acid was the only detected acid and contributed to the overall acid VOC group. Interestingly, acetic acid was not detected in 'BM' or in Group I apples of 'RB' and Group II apples of 'TO'.

The terpene group of VOCs also exhibited variability among cultivars, with α -farnesene being the predominant compound in this group.

Aldehydes were the most abundant VOC group in the flesh of all studied cultivars, with n-hexanal and 2-E-hexenal being the most prevalent compounds. In general, aldehyde content was significantly higher in Group II apples compared to Group I, although the distribution was cultivar-dependent. There was no consistent pattern between fruit sizes across cultivars. Hexyl butyrate and hexyl acetate were the major VOCs in the ester group, while butyl alcohol was the most abundant alcohol. Group II apples exhibited lower alcohol content, except for cultivar 'TO', where alcohol content was higher in Group I apples. Ketones and acids were absent in 'BM', and their content varied inconsistently across fruit sizes in the other cultivars. Acetic acid, the only acid detected, and 2-propanone, the only ketone identified, were present in the flesh of all cultivars. As in the skin, aldehydes (particularly n-hexanal) and α -farnesene were the most abundant VOCs in the flesh. In the terpene group, no consistent differences were found between larger and smaller fruits, with results varying among cultivars.

Table 7. Contents of volatile organic compounds ($\mu\text{g/kg}$ FW) in the skin of five different apple cultivars (‘BM’, ‘RB’, ‘CAR’, ‘OP’, and ‘TO’) of two sizes (Groups I and II).

Volatile Compounds	CAS	RT	‘BM’ I	‘BM’ II	‘RB’ I	‘RB’ II	‘CAR’ I	‘CAR’ II	‘OP’ I	‘OP’ II	‘TO’ I	‘TO’ II
Esters												
Ethyl-butylate	105-54-4	7.259	nd	nd	150.17 $\pm$ 5.11	136.28 $\pm$ 12.94	670.40 $\pm$ 28.99	775.97 $\pm$ 23.60 *	nd	nd	nd	nd
Ethyl 2-methylbutanoate	7452-79-1	7.722	nd	nd	nd	nd	180.01 $\pm$ 17.99	335.33 $\pm$ 30.62 *	nd	nd	nd	nd
Butyl-acetate	123-86-4	8.335	87.52 $\pm$ 7.62	nd	nd	nd	nd	nd	858.43 $\pm$ 26.40 *	300.88 $\pm$ 22.86	nd	nd
2-methylbutyl-acetate	624-41-9	9.948	nd	nd	nd	nd	nd	nd	397.19 $\pm$ 20.36 *	234.41 $\pm$ 21.54	nd	nd
Pentyl-acetate	628-63-7	11.769	nd	nd	nd	nd	nd	nd	131.26 $\pm$ 12.5	nd	nd	nd
Butyl 2-methylbutyrate	15706-73-7	13.806	102.25 $\pm$ 3.48	108.35 $\pm$ 9.48	nd	nd	118.11 $\pm$ 10.42 *	65.99 $\pm$ 4.49	175.24 $\pm$ 10.67	282.41 $\pm$ 17.55 *	142.54 $\pm$ 11.44 *	50.56 $\pm$ 2.77
Ethyl-hexanoate	123-66-0	13.893	nd	nd	nd	nd	158.51 $\pm$ 3.32	238.65 $\pm$ 5.37 *	nd	nd	nd	nd
Hexyl-acetate	142-92-7	15.256	nd	nd	128.15 $\pm$ 12.05 *	73.39 $\pm$ 6.77	140.02 $\pm$ 17.56	nd	3509.73 $\pm$ 278.30 *	612.83 $\pm$ 24.74	nd	nd
Pentyl-butylate	540-18-1	16.81	nd	nd	nd	nd	nd	nd	114.75 $\pm$ 19.83	nd	49.43 $\pm$ 5.99	63.42 $\pm$ 4.37 *
Hexyl-propanoate	2445-76-3	17.598	nd	nd	nd	nd	nd	nd	340.75 $\pm$ 23.71 *	139.24 $\pm$ 14.56	nd	nd
Butyl hexanoate	626-82-4	20.1	618.17 $\pm$ 14.76 *	488.22 $\pm$ 10.65	68.24 $\pm$ 3.77	73.68 $\pm$ 7.71	182.91 $\pm$ 15.52 *	103.42 $\pm$ 3.93	596.63 $\pm$ 20.31 *	409.39 $\pm$ 39.25	505.50 $\pm$ 29.99 *	436.24 $\pm$ 16.00
Hexyl-butylate	2639-63-6	20.15	961.61 $\pm$ 57.63	908.83 $\pm$ 41.07	215.88 $\pm$ 13.43	371.81 $\pm$ 21.10 *	336.23 $\pm$ 26.21 *	247.71 $\pm$ 15.52	1504.98 $\pm$ 120.21 *	757.50 $\pm$ 50.11	1050.03 $\pm$ 41.96	1060.72 $\pm$ 55.04
Hexyl 2-methylbutanoate	10032-15-2	20.513	162.78 $\pm$ 15.38 *	127.83 $\pm$ 12.68	252.97 $\pm$ 19.45	489.99 $\pm$ 29.14 *	345.67 $\pm$ 16.29 *	290.33 $\pm$ 16.66	1057.83 $\pm$ 38.93	1238.38 $\pm$ 151.28 *	72.92 $\pm$ 9.75	90.31 $\pm$ 5.35 *
Hexyl-hexanoate	6378-65-0	26.346	612.52 $\pm$ 18.98 *	407.65 $\pm$ 16.58	89.60 $\pm$ 4.70	126.01 $\pm$ 11.45 *	229.14 $\pm$ 13.32 *	177.28 $\pm$ 14.44	840.42 $\pm$ 11.26 *	447.09 $\pm$ 27.45	311.12 $\pm$ 16.38 *	258.82 $\pm$ 19.34
Butyl-octanoate	589-75-3	26.474	117.33 $\pm$ 9.11 *	78.23 $\pm$ 2.27	nd	nd	nd	nd	76.50 $\pm$ 5.92 *	60.16 $\pm$ 6.29	nd	nd
Alcohols												
Butyl alcohol	71-36-3	10.915	1449.90 $\pm$ 133.36	1386.53 $\pm$ 119	419.73 $\pm$ 26.96	533.48 $\pm$ 33.91 *	415.64 $\pm$ 38.12	502.33 $\pm$ 13.45 *	426.67 $\pm$ 15.78	519.54 $\pm$ 12.97 *	785.36 $\pm$ 25.48	737.19 $\pm$ 29.90
2-methyl-1-butanol	137-32-6	13.046	142.88 $\pm$ 11.19	130.48 $\pm$ 12.23	81.04 $\pm$ 4.41	131.50 $\pm$ 13.54 *	177.98 $\pm$ 17.09	310.77 $\pm$ 18.82 *	110.50 $\pm$ 6.37	209.64 $\pm$ 19.11 *	121.78 $\pm$ 6.96 *	60.93 $\pm$ 4.66
1-Penten-3-ol	616-25-1	11.431	67.15 $\pm$ 2.88	100.61 $\pm$ 9.88 *	53.70 $\pm$ 3.37 *	44.44 $\pm$ 2.00	71.78 $\pm$ 3.71	98.73 $\pm$ 8.52 *	84.17 $\pm$ 7.49	92.00 $\pm$ 7.20	128.50 $\pm$ 8.17 *	96.01 $\pm$ 8.58
2-E-hexen-1-ol	928-95-0	20.016	nd	nd	62.15 $\pm$ 6.41	7.55 $\pm$ 4.55	100.98 $\pm$ 4.65	90.38 $\pm$ 9.43	nd	nd	171.79 $\pm$ 16.37	nd
Aldehydes												
<i>n</i> -hexanal	66-25-1	8.622	7049.62 $\pm$ 169.23	10488.15 $\pm$ 163.01 *	1480.19 $\pm$ 128.46	2512.21 $\pm$ 109.21 *	6082.80 $\pm$ 277.21	7718.34 $\pm$ 144.06 *	4589.16 $\pm$ 188.55	5735.70 $\pm$ 147.84	5537.40 $\pm$ 171.11	5373.92 $\pm$ 113.90
<i>cis</i> -3-hexenal	6789-80-6	10.542	64.41 $\pm$ 5.88	109.27 $\pm$ 4.39 *	73.25 $\pm$ 7.59	94.37 $\pm$ 15.81	112.97 $\pm$ 9.19	154.74 $\pm$ 9.82 *	nd	nd	136.38 $\pm$ 14.87 *	114.59 $\pm$ 6.43
<i>cis</i> -3-hexenal isomer 2	4440-65-7	10.733	nd	nd	nd	nd	95.66 $\pm$ 9.45 *	79.49 $\pm$ 7.97	211.18 $\pm$ 24.09 *	124.47 $\pm$ 25.67	92.21 $\pm$ 8.03 *	58.94 $\pm$ 5.62
2-Z-hexenal	16635-54-4	12.805	151.08 $\pm$ 15.96	137.96 $\pm$ 15.00	127.84 $\pm$ 11.57	148.25 $\pm$ 12.65	147.01 $\pm$ 8.43	208.59 $\pm$ 13.68 *	135.83 $\pm$ 11.06	137.85 $\pm$ 13.63	181.13 $\pm$ 16.04	171.57 $\pm$ 14.05
2-E-hexenal	6728-26-3	13.362	10182.55 $\pm$ 387.95	10886.41 $\pm$ 464.84	7552.79 $\pm$ 170.44	9020.79 $\pm$ 468.61 *	13115.09 $\pm$ 310.78	11867.12 $\pm$ 410.79 *	10897.12 $\pm$ 515.26 *	9459.04 $\pm$ 311.19	12870.77 $\pm$ 382.59 *	14880.89 $\pm$ 486.61 *
Ketones												
2-propanone	67-64-1	2.801	598.01 $\pm$ 35.75	1546.94 $\pm$ 110.79 *	1437.23 $\pm$ 114.88	1701.68 $\pm$ 128.64 *	nd	1708.80 $\pm$ 166.44	2384.38 $\pm$ 186.48	nd	1740.13 $\pm$ 167.60 *	765.34 $\pm$ 150.09
Penten-3-one	1629-58-9	6.88	203.32 $\pm$ 21.20 *	138.17 $\pm$ 14.95	126.24 $\pm$ 17.10	nd	205.69 $\pm$ 26.92	226.37 $\pm$ 18.53	191.02 $\pm$ 18.71 *	99.85 $\pm$ 9.37	194.55 $\pm$ 23.71	265.82 $\pm$ 26.20 *
Methyl heptenone	110-93-0	17.658	71.52 $\pm$ 6.98	136.46 $\pm$ 8.34 *	nd	nd	nd	nd	nd	nd	nd	nd

Acids												
Acetic Acid	64-19-7	2,315	nd	nd	nd	321.21 ± 36.30	118.22 ± 4.71	604.25 ± 16.51 *	462.51 ± 23.99 *	85.45 ± 5.19	105.29 ± 4.63	nd
Terpenes and alkanes												
<i>n</i> -hexane	110-54-3	1,688	157.77 ± 6.28 *	112.7 ± 6.62	89.74 ± 6.72	85.77 ± 6.62	139.69 ± 10.55	183.60 ± 11.83 *	121.17 ± 12.72	185.31 ± 18.86 *	179.21 ± 17.08 *	141.89 ± 4.09
α -Farnesene	502-61-4	30,267	3130.13 ± 139.80 *	2895.42 ± 19.45	1302.79 ± 28.37	1998.85 ± 99.65 *	3885.88 ± 194.24 *	3193.28 ± 40.03	2095.14 ± 183.97 *	1447.18 ± 100.86	2630.47 ± 106.87	3079.33 ± 186.69 *
Total esters			2512.79 ± 216.34 *	2119.16 ± 78.12	876.79 ± 83.27	1246.63 ± 152.87 *	2315.99 ± 218.89	2234.68 ± 244.92	9481.55 ± 762.43 *	4572.29 ± 598.68	2075.45 ± 271.63	2004.35 ± 107.88
Total alcohols			1615.16 ± 251.16	1550.54 ± 143.31	615.10 ± 47.90	713.78 ± 75.43	742.46 ± 58.15	1002.20 ± 40.23 *	621.35 ± 74.71	882.61 ± 92.28 *	1150.16 ± 62.78 *	873.82 ± 92.47
Total aldehydes			17375.825 ±	21635.921 ± 1142.81	9207.08 ±	11739.19 ± 601.84	19521.64 ±	20002.08 ±	15833.81 ±	15484.17 ±	18772.43 ±	20599.92 ± 876.46
			1612.37	*	984.99	*	2085.99	2414.61	1186.78	1075.73	1896.08	
Total ketones			872.85 ± 55.37	1821.57 ± 187.49 *	1563.47 ± 159.67	1701.68 ± 128.64	205.69 ± 26.92	1935.69 ± 178.13	2575.40 ± 117.09 *	99.85 ± 9.37	1934.69 ± 190.73	1031.16 ± 109.24
Total acids			nd	nd	nd	321.21 ± 36.30	118.22 ± 4.71	604.25 ± 16.51 *	462.51 ± 23.99 *	85.45 ± 5.19	105.29 ± 4.63	nd
Total terpenes			3287.92 ± 246.07	3008.12 ± 15.96	1392.53 ± 98.37	2084.62 ± 99.65 *	4025.57 ± 408.84	3376.87 ± 39.77	2216.31 ± 272.31 *	1632.50 ± 102.01	2809.68 ± 175.63	3221.23 ± 210.32 *

Presented data are means ± standard error. Results marked with asterisk (*) represent statistically significant differences ($p < 0.05$) between the same cultivar from Groups I (70+ mm) and II (55–70 mm). No asterisk means there are no statistically significant differences between the same cultivar from Groups I and II. nd: not detected (t -test, $p < 0.05$).

Table 8. Contents of volatile organic compounds ($\mu\text{g/kg}$ FW) in the flesh of five different apple cultivars (‘BM’, ‘RB’, ‘CAR’, ‘OP’, and ‘TO’) of two sizes (Groups I and II).

Aromatics	CAS	Rt	‘BM’ I	‘BM’ II	‘RB’ I	‘RB’ II	‘CAR’ I	‘CAR’ II	‘OP’ I	‘OP’ II	‘TO’ I	‘TO’ II
Esters												
Ethyl-butyrate	105-54-4	7.259	nd	nd	0.27 ± 0.16	0.39 ± 0.16	0.37 ± 0.03	0.42 ± 0.16	nd	nd	nd	nd
Ethyl 2-methylbutanoate	7452-79-1	7.722	nd	nd	nd	nd	8.37 ± 2.35	16.81 ± 2.99 *	nd	nd	nd	nd
Butyl-acetate	123-86-4	8,335	nd	nd	nd	nd	nd	nd	131.66 ± 12.83	141.45 ± 14.52	nd	nd
2-methylbutyl-acetate	624-41-9	9,948	nd	nd	nd	nd	nd	nd	29.39 ± 2.82	58.50 ± 3.14 *	nd	nd
Pentyl-acetate	628-63-7	11,76	nd	nd	nd	nd	nd	nd	16.14 ± 2.92 *	12.17 ± 1.68	nd	nd
Ethyl-hexanoate	123-66-0	13,893	nd	nd	16.53 ± 1.58 *	10.59 ± 1.48	9.79 ± 1.51	16.34 ± 0.67 *	nd	nd	nd	nd
hexyl-butyrate	2639-63-6	20,15	11.87 ± 1.13 *	8.49 ± 1.63	22.73 ± 2.23	28.93 ± 2.07 *	6.36 ± 0.89	nd	72.75 ± 3.62 *	41.14 ± 4.59	39.35 ± 1.28	41.63 ± 4.09
Hexyl-acetate	142-92-7	15,256	nd	nd	9.90 ± 1.24 *	5.59 ± 0.96	8.31 ± 1.15	9.84 ± 1.01	493.91 ± 29.94 *	360.22 ± 28.53	5.96 ± 0.74	nd
Hexyl 2-methylbutyrate	10032-15-2	20,513	4.50 ± 1.29	8.27 ± 1.71 *	nd	nd	nd	nd	8.93 ± 2.28	12.98 ± 2.67	nd	nd
Alcohols												
Butyl alcohol	71-36-3	10,915	75.17 ± 2.98	108.51 ± 11.77 *	47.26 ± 4.77	82.07 ± 6.81 *	43.44 ± 4.39	52.36 ± 2.67 *	39.17 ± 3.10	52.12 ± 5.03 *	70.99 ± 7.49 *	56.12 ± 1.13
2-methyl-1-butanol	137-32-6	13,046	5.77 ± 0.85	9.22 ± 0.75 *	nd	12.46 ± 1.90	18.08 ± 2.81	28.60 ± 2.07 *	n.d.	13.33 ± 1.61	4.74 ± 0.47	nd
Aldehydes												
Butanal	123-72-8	3,566	14.30 ± 1.39	16.40 ± 1.94	nd	9.13 ± 1.81	9.40 ± 0.94	nd	nd	nd	10.99 ± 1.17	8.73 ± 1.41
<i>n</i> -hexanal	66-25-1	8,622	145.94 ± 11.89	313.77 ± 12.27 *	76.56 ± 5.77	137.43 ± 11.77 *	185.95 ± 10.10	279.89 ± 11.44 *	174.80 ± 8.65	388.17 ± 14.63 *	109.46 ± 6.81	150.18 ± 0.28 *
2- <i>E</i> -hexenal	6728-26-3	13,362	136.52 ± 14.05	207.99 ± 11.25 *	109.21 ± 10.68	195.23 ± 11.80 *	200.89 ± 13.84	202.31 ± 17.68	255.67 ± 10.08	373.76 ± 20.83 *	213.61 ± 14.64	272.01 ± 16.42 *
Ketones												
2-propanone	67-64-1	2,801	nd	nd	37.01 ± 3.76 *	22.96 ± 1.22	23.52 ± 2.24	49.60 ± 4.23 *	43.29 ± 2.19	37.29 ± 4.81	103.91 ± 11.86 *	84.08 ± 7.53
Acids												
Acetic acid	64-19-7	2,315	nd	nd	28.92 ± 0.91 *	18.99 ± 1.18	25.19 ± 2.21	35.53 ± 1.93 *	19.32 ± 1.87	27.74 ± 1.85 *	nd	nd

Terpenes and alkanes

<i>n</i> -hexane	110-54-3	1,688	14.14 ± 1.70	15.91 ± 0.72	28.47 ± 1.45	27.75 ± 2.43	640.87 ± 14.89 *	390.43 ± 19.36	42.34 ± 4.50	41.92 ± 4.50	24.40 ± 2.64	31.41 ± 6.39 *
D-Limonene	5989-27-5	12,572	nd	nd	nd	nd	nd	nd	4.52 ± 1.46	4.12 ± 1.51	nd	nd
α -Farnesene	502-61-4	30,267	28.45 ± 2.74	46.86 ± 1.12 *	8.06 ± 1.95	10.59 ± 2.17	18.12 ± 2.45	17.98 ± 1.35	13.13 ± 2.09	10.70 ± 2.13	19.11 ± 0.29 *	14.46 ± 1.19
Total esters			16.87 ± 1.67	16.00 ± 0.97	47.02 ± 1.17	45.46 ± 3.20	33.20 ± 3.52	43.25 ± 4.39 *	752.79 ± 67.57 *	626.46 ± 30.88	45.23 ± 6.14	41.63 ± 4.09
Total alcohols			80.93 ± 2.48	117.72 ± 20.68 *	51.48 ± 5.04	94.53 ± 6.83 *	61.52 ± 8.20	80.96 ± 4.11 *	39.17 ± 3.10	65.45 ± 8.28 *	74.14 ± 7.70 *	56.12 ± 1.14
Total aldehydes			291.99 ± 31.98	532.69 ± 42.63 *	185.78 ± 20.45	338.74 ± 37.84 *	393.10 ± 25.87	482.20 ± 50.08 *	430.47 ± 43.46	761.93 ± 82.56 *	326.73 ± 37.44	430.92 ± 48.42 *
Total ketones			nd	nd	37.01 ± 3.76 *	22.96 ± 1.22	23.52 ± 2.24	49.60 ± 4.23 *	43.29 ± 2.19	37.29 ± 4.81	103.91 ± 11.86 *	84.08 ± 7.53
Total acids			nd	nd	28.92 ± 0.91 *	18.99 ± 1.18	25.19 ± 2.21	35.53 ± 1.93 *	19.32 ± 1.87	27.74 ± 1.85 *	nd	nd
Total terpenes			42.59 ± 4.75	62.77 ± 5.92 *	36.53 ± 1.69	38.34 ± 0.82	658.99 ± 62.89 *	408.41 ± 46.20	59.06 ± 1.75	54.79 ± 6.88	39.84 ± 0.71	45.87 ± 2.49 *

Presented data are means ± standard error. Results marked with asterisk (*) represent statistically significant differences ($p < 0.05$) between the same cultivar from Groups I (70+ mm) and II (55–70 mm). No asterisk means there are no statistically significant differences between the same cultivar from Groups I and II. nd: not detected (t -test, $p < 0.05$).

4. Discussion

Fruit quality is determined by a variety of parameters, encompassing both external and internal characteristics. One key quality marker for apples is fruit size, as there are established size standards that classify apples as either marketable or non-marketable. Non-marketable apples may still be utilized for processing purposes. Taste, another significant factor, affects both the quality of the fruit and consumer acceptance [21]. The two primary taste profiles of apples—sour and sweet—are determined by the sugar-to-acid ratio [22].

Our findings, based on flesh firmness, color, soluble solid (SS) content, and starch-iodine testing, suggest that Group I (≥ 70 mm) and Group II (55–70 mm) apples exhibit uniform ripeness. Despite this uniformity in ripeness, notable differences in sugar and organic acid content were observed, highlighting the influence of fruit size on quality. The cultivar ‘CAR’ was the only variety in which a greater total sugar content was observed in Group II (smaller apples), whereas ‘RB’ showed no significant differences in total sugar content between the larger and smaller apples. The cultivars ‘TO’, ‘BM’, and ‘OP’ exhibited higher total sugar content in smaller apples (Group II), suggesting a water dilution effect, where total sugar concentration decreases as fruit size increases. These results underscore that both fruit size and genotype contribute to variations in sugar content [23].

The sugar-to-acid ratio plays a crucial role in determining the flavor profile of apples [24]. Our results are consistent with those of Wang [25], where the sugar-to-acid ratio ranged from 5.4 to 17.5, with higher ratios indicating sweeter apples. While no statistically significant differences in the sugar-to-acid ratios were found between Group I and Group II apples within the same cultivar—except for a higher ratio in the skin of larger ‘BM’ apples—differences in the composition of sucrose and fructose were apparent. These differences suggest that fruit size influences the distribution of specific sugars, primarily sucrose and fructose, without significantly altering the sweetness–acidity perception. The sweetness index did not always correlate directly with the sugar-to-acid ratio (‘CAR’ II flesh, ‘OP’ I skin, ‘OP’ I flesh, ‘TO’ I flesh), indicating that apple sweetness may be more closely related to the composition of individual sugars rather than the total sugar-to-acid ratio. This finding aligns with Aprea et al. [26], who reported that variations in sucrose, glucose, fructose, and sorbitol concentrations can influence sweetness perception, even when the overall sugar-to-acid ratio remains constant across cultivars.

Phenolic compound content in the skin and flesh of apples also varied by cultivar and fruit size. Total flavanol content was the predominant group of phenolic compounds, particularly in the skin and flesh of white-fleshed cultivars, as previously reported by De La Iglesia et al. [27]. Our findings suggest that the total phenolic compound content and its distribution are cultivar-dependent. This observation is supported by Liang et al. [28], who found notable differences in the phenolic profiles of 35 apple cultivars. In the flesh of ‘RB’, ‘CAR’, ‘OP’, and ‘TO’, the total analyzed phenolic content (TAPC) was significantly higher in smaller apples (Group II), likely due to higher concentrations of hydroxycinnamic acid, dihydrochalcones, and flavanols. These results are in agreement with Busatto et al. [1], who observed a negative correlation between fruit weight and phenolic content in the flesh, while the skin exhibited cultivar-specific differences. The dilution effect—where a larger fruit size results in a lower concentration of phenolic compounds due to cell enlargement—is a plausible explanation for these findings. However, this effect was not observed in the red-fleshed cultivar ‘BM’, which showed similar TAPC levels across fruit sizes, which was mainly driven by high flavanol content in the flesh of both bigger and smaller fruits. This result may be due to increased phenolic bioavailability, mainly through the action of the MYB10 transcription factor, which activates anthocyanin biosynthesis through anthocyanin-related genes and reduces the impact of fruit size on phenolic

concentration compared with white-fleshed cultivars [1]. The lack of phenolic accumulation in 'BM' flesh, compared to white-fleshed cultivars, may be attributed to the generally lower phenolic content in red-fleshed apples (primarily in the flesh). The higher anthocyanin content in red-fleshed apples, as reported by Espley et al. [29–31] and Busatto et al. [1], could account for the lower flavanol concentrations in these cultivars due to competitive biosynthesis pathways, where both anthocyanins and flavanols share common precursors.

Volatile organic compounds (VOCs) are another key group of secondary metabolites influencing the aroma and organoleptic quality of fruits [32]. In line with previous studies [33,34], we found that aldehydes were the most abundant VOCs in the skin and flesh of the studied apple cultivars, with some cultivars showing significantly higher aldehyde concentrations compared to other VOCs. In contrast, acids, ketones, and terpenes were not detected in all cultivars. Acids were absent from the skin of 'BM' (both Groups I and II), 'RB' Group I, and 'TO' Group II, while ketones were absent from 'BM' across both fruit sizes. In cultivars where acids were undetected in the skin (e.g., 'RB' Group I and 'TO' Group II), Group II apples of 'RB' and 'TO' exhibited significantly higher ester content, indicating that esterification of the acids had occurred. The major esters, alcohols, aldehydes, ketones, acids, and terpenes detected in our study have been previously identified as key aromatic compounds in apples [21].

Aldehydes, particularly n-hexanal and 2-E-hexenal, are responsible for the characteristic green apple, freshly cut grass, and unripe fruit aromas [35]. Alcohols, such as butyl alcohol and 2-methyl-1-butanol, contribute to mild, fruity, sweet, and pungent apple flavors [36], while ketones like 2-propanone and penten-3-one add floral, fruity, and sweet notes [37]. Acetic acid was the only acid detected in both skin and flesh, consistent with findings by Liu et al. [38], who noted that acetic acid is typically not a dominant VOC in apples but may form during oxidative processes. Moreover, in ripe apples, esters are the most abundant group of VOCs; however, our results suggest that ripeness stage (apples were harvested in technological maturity) or genetic background might influence VOC biosynthesis. The study of Liu et al. [38] analyzed the transcriptome profiles of the 'Ruixue' apple cultivar, where aldehydes were also most abundant when ripe, and its parental lines throughout fruit development and identified numerous genes associated with aroma biosynthesis, including those involved in fatty acid, isoleucine, and sesquiterpenoid metabolic pathways, as well as transcription factors that may play regulatory roles in the production of aroma compounds. The study of Espino-Díaz et al. [8] also found that aldehydes are transformed to esters under regular atmosphere; however, under controlled atmosphere, the increased CO₂ causes a change in ADH (alcohol dehydrogenase) activity, which results in a reduction in alcohol and therefore limits the production of esters. Terpenes, especially α -farnesene, contribute to the ripening and fresh, fruity, floral, and citrusy aromas of apples [39]. Interestingly, D-limonene, a compound prominent in citrus fruits, was detected in the flesh of both Group I and Group II apples of cultivar 'OP', enhancing the overall citrusy and fruity notes.

While esters are recognized as the primary contributors to apple flavor, comprising over 80% of total volatile compounds in varieties such as 'Granny Smith', 'Gala', and 'Starking Delicious' [40–42], our results indicate that aldehydes were the dominant VOCs in all studied cultivars. This finding aligns with previous studies, such as those by Yang et al. [43], where aldehydes accounted for up to 69.23% of the total volatile content in 'Cox Orange' apples. To fully assess the impact of these aldehydes on apple flavor, further research and sensory evaluations are necessary.

Overall, no consistent differences in VOC profiles were observed between small and large apples within the same cultivar, suggesting that these differences are more likely cultivar-specific rather than size-dependent. The uniformity of VOC expression across

different fruit sizes may reflect a consistent biosynthetic gene expression in the fruit tissues, independent of size, or the size-independent partitioning of aroma compounds [44].

5. Conclusions

Based on measurements of various physical and chemical parameters, including sun-exposed and shaded-side fruit color, fruit firmness, soluble solid (SS) content, diameter, starch-iodine test, and weight, the uniformity of differently sized fruits was confirmed. No statistically significant differences were observed for any physical parameter, except for weight and size, which aligns with the focus of our study on fruit size only.

With the exception of the red-fleshed cultivar 'BM', the sugar/acid ratio did not differ between fruit sizes of the same cultivar. However, the composition of individual sugars and organic acids varied between apple sizes, depending on the cultivar, thus influencing the sweetness index. This suggests that the composition of individual sugars is more critical than the sugar/acid ratio in determining the overall sweetness profile.

In this study, the total anthocyanin and phenolic content (TAPC) in the flesh was significantly higher in the smaller (Group II) white-fleshed apple fruits compared to the larger (Group I) apples of the same cultivar. This could be attributed to the dilution effect, where larger fruits may have lower concentrations of certain biochemical compounds due to cell expansion, which outpaces the biosynthesis or accumulation of these compounds, leading to decreased content per unit of fresh weight. No consistent differences in TAPC were observed between Group I and II apple fruits in the skin.

There were no significant differences in total organic acid content between Groups I and II apple fruits in the skin and flesh for all studied cultivars, except for 'OP', where higher total organic acid content was found in the flesh of Group II apples.

Overall, fruit size did not appear to influence the fruit aroma profile (volatile organic compounds, VOCs) or the sugar and organic acid content. Aldehydes were the dominant group of VOCs in both the skin and flesh of all five cultivars, which could be explained by the technological maturity of the harvest, where the conversion of aldehydes into alcohols and therefore esters did not fully happen. The composition and total content of VOCs, however, varied significantly between fruit sizes, indicating cultivar-specific patterns. In conclusion, the impact of fruit size on quality does not occur uniformly across cultivars; rather, it is cultivar-dependent. Therefore, a metabolic study should be conducted for each cultivar before determining the suitability of smaller (Group II) fruits for either fresh consumption or further processing. Such studies would help identify the appropriate quality attributes for each cultivar. Future work should be conducted to understand cultivar-specific size effects, such as transcriptomic analysis to explore gene regulation and environmental effects on VOCs.

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