Metabolomic Profiling and Sensorial Quality of 'Golden Delicious', 'Liberty', 'Santana', and 'Topaz' Apples Grown Using Organic and Integrated Production Systems

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Ⓢ *Supporting Information*

ABSTRACT: Apple quality was investigated in the scab-resistant 'Liberty', 'Santana', and 'Topaz' cultivars and the scab-susceptible 'Golden Delicious' cultivar. Trees subjected to the same crop load were cultivated using either an organic (ORG) or an integrated production (IP) system. Physicochemical properties, phenolic content, and sensorial quality of fruit from both systems were compared. There were no significant differences in fruit mass, starch, and total soluble solid content (the latter was higher in ORG 'Liberty') between ORG and IP fruit, whereas significantly higher flesh firmness was found in ORG fruit (except no difference in 'Golden Delicious'). Significantly higher total phenolic content in ORG fruit was found in 'Golden Delicious', whereas differences in other cultivars were not significant. Targeted metabolomic profiling of multiple classes of phenolics confirmed the impact of the production system on the 'Golden Delicious' phenolic profile as higher levels of 4-hydroxybenzoic acid, neo- and chlorogenic acids, phloridzin, procyanidin B2+B4, -3-*O*-glucoside and -3-*O*-galactoside of quercetin, kaempferol-3-*O*-rutinoside, and rutin being found in ORG fruit. The results obtained suggested that scab resistance influenced the phenolic biosynthesis in relation to the agricultural system. Sensorial evaluation indicated significantly better flavor (except for 'Topaz') and better appearance of IP fruit.

KEYWORDS: *apples, organic production, integrated production, scab resistance, UPLC-MS/MS, targeted metabolomic profiling, polyphenols, sensorial quality*

INTRODUCTION

Fruits are the biggest source of phenolics, the most widespread plant micronutrients, important for human health and providing effective antioxidant, anti-inflammatory, vasodilatory, and prebiotic properties.[1] According to the Food and Agriculture Organization (FAO), 75.6 million tons of apples was grown worldwide in 2011.[2] As they are produced in such large quantities, apples are an important source of phenols, especially proanthocyanidins, in the human diet.[3,4] The main phenolic classes in apples are flavanols (catechins and proanthocyanidins), followed by hydroxycinnamates, flavonols, dihydrochalcones, and red apple anthocyanins.[5,6] Polyphenols have been found to be the main source of antioxidants in apples, rather than vitamin C.[7] The main contributors to the antioxidant activity of apples have been found to be flavan-3-ols/procyanidins,[8] in terms of the five major phenolic groups, and procyanidin B2, quercetin, and epicatechin in terms of individual compounds.[7,8]

Besides their importance for human health, phenols are an important factor in terms of plant resistance to pathogens, herbivores, and other biotic and abiotic stress factors.[9] They play an important role in the resistance of apple trees to scab fungus *Venturia inaequalis*,[10] which is the most widespread disease in apple-growing areas with high spring and summer rainfall. There are reports that higher contents of different flavan-3-ols, hydroxycinnamates, and flavonols has been found

in the tissue of leaves and fruit infected with *V. inaequalis* in comparison to healthy tissue.[11]

Disease control in commercial orchards can require up to 15 fungicide treatments per year. Due to the ecological damage caused by pesticides and synthetic fertilizers, the organic (ORG) system has been adopted as an alternative to conventional or integrated production (IP). It has been evaluated that the ORG system ranks first in terms of environmental and economic sustainability, the IP system second (the most persistent pesticides excluded from use), and the conventional system last (wider list of allowed pesticides, mostly thought of as full/complete chemical plant protection).[12] Until today, apple production in Europe has mostly been managed according to IP guidelines; however, the quantity of ORG-produced apples is increasing constantly at the global level.[13,14] The reason is consumer conviction that ORG apples contain more bioactive compounds and cause less environmental problems than IP.[15] Indeed, there are reports of higher phenolic compound content in ORG-grown apples.[16,17] It was also found that crop load per tree was inversely correlated to the phenolic content in apple fruit.[18] Considering the commonly smaller yield per hectare of ORG-produced

apples, crop load may be one of the factors confusing estimated differences in phenolic content between ORG and IP production managements. On the other hand, there are reports suggesting the effect of agricultural practice on phenolic content and antioxidant capacity in different apple varieties is not significant.[19−21] According to Zhao et al.,[15] the overall evidence seems to be in favor of enhancement of phytochemical content in ORG-grown produce, but there have been few systematic studies of the factors that may contribute to increased phytochemical content in ORG crops.

The aim of this study was to investigate how the ORG or IP agricultural system affects both physicochemical quality parameters and the phenolic content of fruit. Four popular apple cultivars, frequently planted in Europe, were investigated: 'Golden Delicious', 'Liberty', 'Santana', and 'Topaz'. The characteristics of the cultivars chosen were as follows: 'Golden Delicious' is an old and widespread apple scab-susceptible cultivar[10] and is the most widely produced cultivar in European Union (EU) countries.[22] 'Liberty' is an old scab-resistant cultivar popular in America,[23] 'Topaz' is a new scab-resistant cultivar frequently used in ORG production in Central Europe,[24,25] and 'Santana' is a new scab-resistant and also hypoallergenic cultivar.[26] Different groups of apple phenols were determined, together with antioxidant capacity. Furthermore, phenolics within multiple classes were evaluated to understand which phenols were significantly affected by ORG/IP production management. The results obtained were then compared statistically to evaluate significance. Apples from both production systems were subjected to sensory evaluation by eight panelists to assess flavor and general appearance.

■ MATERIALS AND METHODS

Plant Material. The experiment was conducted in 2010. Seven-year-old slender spindle apple trees (*Malus × domestica* Borkh.) on dwarf M.9 rootstocks were grown in the experimental orchard of the Agricultural Institute of Slovenia in Brdo (latitude, 46° 10′ N; longitude, 14° 41′ E). In the year 2010 there was hotter weather in comparison to the average of the past 30 years. Until the harvest time (September 22) the effective temperature sum (above 8 °C) was 1602 °C, whereas the rainfall sum was 577 mm. All four cultivars were planted using two production systems, each in one section of the same field at a distance of 10 m between them. From the orchard establishment one section was run according to the Guidelines for Integrated Production of Pome Fruits in Europe[28] and the other according to ORG system under European Union Regulation No. 2092/91.[29] Briefly, the IP managed section was sprayed below the crowns with glyphosate to maintain a clear herbicide strip, whereas ORG-grown trees were mechanically cleaned below the crowns. In the year of experiment no chemical thinning of fruitlets and no fertilization were performed on either the IP or ORG sections. Contact and systemic fungicides or insecticides allowed in IP management were used in the IP section (twice copper fungicide, four times lime sulfur, twice cyprodinil, twice difenoconazole, twice mancozeb, three times dithianon, four times captan, once dodine, twice organophosphorus esters). ORG-grown trees were sprayed with copper products (eight times) and lime sulfur (eight times) as fungicide treatments, whereas azadirachtin-based insecticide (twice) and granulosis virus (eight times) were used against aphids and codling moth, respectively. The IP and ORG experimental plots were in neighboring orchard sections to ensure the microclimate and soil type were the same. In both sections a complete random block design with six replications was set up. Each block was made up of trees of four cultivars: three apple scab-resistant cultivars ('Liberty', 'Santana', and 'Topaz') and one scab-susceptible cultivar ('Golden Delicious'). The statistical unit for the sample supply was one individual tree. The selected trees were homogeneous in terms of flower set, vigor, and health status within each block. In July, hand thinning was carried out and crop load was set to 40 fruits per tree. Trees with a lower crop load were excluded as the sample source. The fruit was harvested at technological maturity, determined by a Streif index of 0.90 (firmness, soluble solids, and starch measurements) on 'Golden Delicious' fruit sampled in the same orchard. The harvest date of 'Liberty' and 'Topaz' was determined according to ground and blush color changes and relative to 'Golden Delicious' appointed harvest date. The harvest date of 'Santana' was based on blush color changes and according to several years of experience. 'Santana' was harvested on September 1, and 'Liberty', 'Topaz', and 'Golden Delicious' were harvested on September 22. The yield of each tree was weighed and counted, and 20 fruits per tree with an equatorial diameter of 70−80 mm were considered as a sample for determination of the physicochemical and sensorial parameters. Apples were stored at 4 °C and at high relative humidity (90−95%) for eight days for 'Santana' and 2 months for the other three cultivars. When apples were removed from storage and ground color was starting to change to yellow, physicochemical analysis and aqueous acetone extracts were prepared, that is, 2 days after storage for 'Santana' and within 1 week after storage for the other three cultivars, respectively. On the following day the sensorial test was performed.

Chemicals and Reagents. Formic acid, methanol, and acetonitrile of LC-MS grade (Sigma-Aldrich, St. Louis, MO, USA) and ultrapure water of Milli-Q gradient (Millipore Corp., Billerica, MA, USA) were used for chromatography. Folin−Ciocalteu and vanillin were from Merck (Darmstadt, Germany). Trolox equivalent antioxidant capacity (TEAC) antioxidant assay kit (product no. CS0790) was from Sigma (St. Louis, MO, USA), and solutions were prepared under instructions. Phenol standards for mass spectrometry analysis were obtained from different suppliers,[27] whereas *cis*-resveratrol and *cis*-piceid were produced by photochemical isomerization of the *trans-* forms.[30]

Determination of Apple Mass, Starch, Soluble Solids, Firmness, Russeting Appearance, and Share of Red Blush Color. Average fruit mass was calculated from the whole tree yield divided by the number of fruits per tree. Eight apples per sample were considered to measure the share of red blush color, russeting appearance, fruit firmness, starch, and soluble solids. First, the skin color on each fruit was estimated visually from 0 (0% red blush) to 10 (100% red blush), along with skin russeting appearance on a scale from 0 (no russeting) to 10 (skin 100% covered by russet). After blush and russeting estimations, destructive measurements on the fruit were performed. A penetrometer mounted on a stand was used to measure flesh firmness, after part of the skin had been removed at four locations along the equator of each apple. Penetration into the fruit cortex was performed with a 11.3 mm standard probe (1 cm^2) using a homemade and calibrated electronic penetrometer equipped with a force sensor, amplifier, and computer with Catman software for data collection (HBM, Darmstadt, Germany).[31] The starch−iodine index was determined by immersing the stem side of eight apple equatorial cross sections in 0.1 M iodine solution, with staining of fruit halves estimated on a 1−10 scale (1 = highest starch content = 100% staining; 10 = no staining, over-ripe fruit). The percentage of soluble solids was measured using a digital refractometer (PAL-1; Atago Inc., Bellevue, WA, USA) with juice obtained during measurement of flesh firmness, and reported as °Brix.

Apple Fruit Extraction. Apples were extracted in aqueous 70% acetone, as described.[18] Briefly, to limit enzymatic and chemical reactions (especially oxidation), both apples and the aqueous acetone were cooled to 4 °C. The pith of six fruits per sample was removed with a corer, and each apple was cut into eight equal slices. Two slices by length section (cortex plus skin) from the opposite side of each fruit were collected, rapidly weighed (mass was approximately 120 g), and homogenized in 250 mL of cold aqueous acetone for 90 s. Homogenates were extracted for 15 min and centrifuged for 5 min at 3600g. Sediments were extracted again in 200 mL of aqueous acetone for 15 min and centrifuged as before. Both supernatants were pooled, and the volume was adjusted to 500 mL. Extracts were placed in dark glass bottles, flushed with nitrogen, and stored at −20 °C until analyzed.

Table 1. Physicochemical Fruit Quality Parameters, Russeting Index, and Red Blush Index of Organic (ORG) and Integrated Production (IP) Systems

cultivar	production system	fruit mass (g)	starch index (1−10)	soluble solids (°Brix)	fruit firmness (N)	russeting index (0−10)	red blush index (0−10)
Golden	ORG (3[a])	166 a[b]	9.3 a	16.8 a	63 a	2.6 a	2.0 a
Delicious	IP (4)	208 a	8.9 a	16.2 a	63 a	0.5 b	1.2 a
Liberty	ORG (4)	144 a	8.6 a	16.7 a	79 a	0.2 a	9.4 a
	IP (4)	154 a	8.7 a	15.9 b	76 b	0.1 a	9.3 a
Santana	ORG (5)	197 a	6.3 a	13.4 a	77 a	1.1 a	6.5 b
	IP (6)	218 a	5.7 a	11.9 a	68 b	0.0 b	7.9 a
Topaz	ORG (5)	167 a	8.9 a	15.6 a	79 a	1.4 a	7.3 a
	IP (5)	166 a	8.5 a	15.7 a	76 b	1.2 a	6.9 a

[a]Number of samples (the statistical unit for the sample was an individual tree). [b]Different letters indicate a significant difference between ORG/IP means in each cultivar with F test at $P \leq 0.05$.

Determination of Total Polyphenols, Low and High Molecular Weight Proanthocyanidins, and Total Anthocyanins. Total polyphenols, low molecular weight proanthocyanidins, high molecular weight proanthocyanidins, and total anthocyanins were determined spectrophotometrically as described.[32] Acetone was removed from 20 mL aliquots of extracts by rotary evaporation under reduced pressure at 35 °C. Samples were then reconstituted with ultrapure water and cleaned up by using 0.5 g C18 SPE columns (Waters, Milford, MA, USA) for each assay as described.[32] A preliminary cleanup of samples was performed to remove polar compounds, such as ascorbic acid, sugars, organic acids, and amino acids, that could cause interference.

Total Polyphenols. Total phenols were assessed by the reduction of phosphotungstic−phosphomolybdic acids (Folin−Ciocalteu reagent) to blue pigments using phenols in alkaline solution. Contents were determined by means of a calibration curve as mg/kg fresh weight (FW) of (+)-catechin.

Low Molecular Weight Proanthocyanidins. Low molecular weight proanthocyanidins were assessed by the index of vanillin, which provides an estimation of the free C6 and C8 of both catechins and proanthocyanidins. Therefore, the method provides a good estimation of free flavanols and low degree of polymerized flavanols. Contents were expressed by means of a calibration curve in mg/kg FW of (+)-catechin.

High Molecular Weight Proanthocyanidins. Polymeric proanthocyanidins were evaluated through their transformation into cyanidin in a boiling water bath by using iron salts in HCl as catalyst to increase the reproducibility of yield of cyanidin and by the use of optimal percentage of ethanol. Concentrations were expressed by means of a calibration curve as mg/kg FW of cyanidin chloride.

Total Anthocyanins. Total anthocyanins were determined on the basis of maximal absorbance in the visible range (536−542 nm). They were quantified in mg/kg FW by assuming an average absorbance of the mixture of anthocyanins extracted from grape Cabernet Sauvignon (average MW = 500 Da, ε = 18800 M^{-1} cm^{-1} in a 70:30:1 ethanol/water/HCl solution).

Determination of Antioxidant Capacity. Antioxidant capacity was determined in apple fruit aqueous acetone extracts using the TEAC antioxidant assay kit. The TEAC assay principle is formation of a ferryl myoglobin radical from metmyoglobin and hydrogen peroxide, which oxidizes ABTS to a radical cation, ABTS$^{\bullet+}$. ABTS$^{\bullet+}$ was determined spectrophotometrically at 405 nm. Antioxidants in the sample suppress the production of ABTS$^{\bullet+}$ in a concentration-dependent manner, and the color intensity decreases proportionally. Antioxidant capacity was determined using standard Trolox in mmol/kg FW of Trolox equivalents.

Acetone was removed from apple fruit extracts using rotary evaporation under reduced pressure at 35 °C, and samples were reconstituted with ultrapure water. ABTS$^{\bullet+}$ was generated in a

microcuvette containing 10 μL of sample, 20 μL of myoglobin working solution, and 150 μL of ABTS substrate working solution. After 5 min of incubation, stop solution was added and absorbance was measured at 405 nm against the corresponding blank. When necessary, the sample was diluted before analysis in assay buffer one time to bring the antioxidant level within the range of the calibration curve. Analyses were prepared in duplicates.

Determination of Multiple Classes of Phenolics. An utraperformance liquid chromatography−tandem mass spectrometry (UPLC-MS/MS) targeted metabolomic method for rapid quantitation of multiple classes of phenolics in fruits and beverages was used for determination of phenolic metabolites in apple acetone−aqueous extracts.[27] The method used an Acquity UPLC connected to a Xevo TQMS (Waters, Milford, MA, USA). Aqueous acetone apple extracts were kept at 6 °C during analysis, and 2 μL was directly injected into a system. Separation was performed on a 100 mm × 2.1 mm, 1.8 μm column (Acquity HSS T3, Waters), maintained at 40 °C. Flow was set to 0.4 mL/min, and mobile phase A was 0.1% formic acid in water, whereas mobile phase B was 0.1% formic acid in acetonitrile, following a linear gradient from 5% B to 20% B in 3 min, isocratic at 20% B for 1.3 min, from 20 to 45% B in 4.7 min, from 45 to 100% B in 2 min, 2 min at 100% B, and in 3 min back to 5% B. Mass spectrometry detection of phenols was performed with electrospray ionization (ESI) in positive and negative modes as described.[27]

Evaluation of Sensorial Parameters. Sensory evaluations by panels were conducted one day after physicochemical analyses. For each cultivar two consecutive blind consumer taste panels were carried out on the same day by eight healthy nontrained participants, informed only generally with regard to the purpose of the study. A quick hedonic test/education was performed just before panel; on 'Gala' as a sample of sweet apple, on 'Idared' as a sample of tart apple, and on 'Cripps Pink' as a sample of a crisp and hard apple. The purpose of first panel was to judge if panelists were capable of differentiating ORG/IP apples, whereas the second panel hedonistically rated the intensity of the most important sensorial parameters of fruit samples. Apples were sensorially evaluated at room temperature. Unpeeled apples were cored and cut into eight equally sized slices (stem to calyx) with an apple corer. Only one cultivar per day was judged.

First, in the triangle test the panelists tasted blind three slices of apple (two from one production system and one from the other) and were asked to identify the slice that was different from the other two. Three individual slices of apple were placed on paper, labeled with three different blind codes, one for each slice, and immediately served to a panelist. For each cultivar, the panelists assessed five triads (five replications) separately in random order. To neutralize taste between replications, bread was provided to panelists.

The second panel, that is, the hedonic/intensity test, immediately followed the first panel. The panelists rated the taste of 10 blind apple

Table 2. Contents of Total Polyphenols (TP), Low Molecular Weight Proanthocyanidins (LWP), High Molecular Weight Proanthocyanidins (HWP), Total Anthocyanins (TA), and Antioxidant Capacity (AC) of Apples Grown Using Organic (ORG) and Integrated Production (IP) Systems

cultivar	production system	TP (mg catechin/kg FW)	LWP (mg catechin/kg FW)	HWP (mg cyanidin/kg FW)	TA (mg/kg FW)	AC (mmol Trolox/kg FW)
Golden	ORG (3[a])	1050 a[b]	864 a	1064 a	ND[c]	11.9 a
Delicious	IP (4)	716 b	592 b	744 b	ND	11.5 a
Liberty	ORG (4)	901 a	717 a	1006 a	45.5 a	8.9 a
	IP (4)	850 a	645 a	828 a	44.5 a	8.6 a
Santana	ORG (5)	592 a	353 a	646 a	19.6 a	7.4 a
	IP (6)	567 a	268 b	494 b	19.0 a	5.8 b
Topaz	ORG (5)	662 a	657 a	792 a	26.0 a	6.7 a
	IP (5)	654 a	693 a	763 a	27.2 a	7.3 a

[a]Number of samples (the statistical unit for the sample was an individual tree). [b]Different letters indicate a significant difference between ORG/IP means in each cultivar with F test at $P \leq 0.05$. [c]ND, not detected.

slices (five ORG and five IP in random order, one slice per block) on a nine-point hedonic scale. Sweetness, tartness, crispness, firmness, and juiciness (1 = not at all sweet/tart/crisp/extremely soft/not at all juicy; 9 = extremely sweet/tart/crisp/hard/juicy) and overall flavor (1 = extreme dislike, 5 = good, 9 = extreme like) were evaluated.

Panelists also rated the overall appearance of ORG- and IP-produced apples, which were presented to them in two identical boxes (containing 12−15 apples). Assessment was done on the same nine-point scale (1 = dislike appearance, 9 = really nice fruits) as the overall appearance fruit quality parameter. As there were no repetitions for fruit overall appearance, statistical calculation of scoring was not possible, so the means of only eight evaluations are presented (Table 4).

Statistical Analysis. The data were analyzed using the Statgraphics Centurion XVI program (Manugistics Inc., Rockville, MD, USA) to provide ANOVA significance of ORG/IP management effects. Significant differences between the means were determined using the F test at $P \leq 0.05$.

■ RESULTS AND DISCUSSION

Apple Mass, Starch, Soluble Solids, Firmness, Russeting Appearance, and Share of Red Blush Color. The basic physicochemical parameters determining apple fruit quality are presented in Table 1. The mass of apples represents the average of the whole yield per tree, and all further parameters were determined in the same-sized apples (70−80 mm) to avoid the impact of size on phytochemical content. There were no significant differences in either fruit mass or starch content between ORG and IP systems for all four varieties investigated, whereas soluble solid content was significantly higher only in the case of ORG 'Liberty' apples, and red blush was significantly lower only in ORG 'Santana' fruit. On the other hand, ORG-grown apples had significantly higher flesh firmness, which is a positive consumer attribute for apples (except in the case of 'Golden Delicious', where on average differences in flesh firmness were not found). There have also been other reports of higher fruit firmness at harvest and after storage for ORG apple fruit in comparison to IP.[12,17,33] Amarante et al.[34] found higher fruit firmness, higher total soluble solid content, and higher russeting in ORG-grown 'Royal Gala' and 'Fuji' apples, but Peck et al.[35] did not find any differences between ORG and IP production systems when the physicochemical parameters of 'Liberty' were measured, such as starch index, soluble solids, titratable acidity, and fruit firmness. In our study ORG-grown apples had slightly more russet on

fruit surface, mostly near the fruit pedicel cavity; however, a statistically significant difference was found only in the case of 'Golden Delicious' (index = 2.6 and 0.5 in ORG and IP fruits, respectively) and 'Santana' (1.1 and 0.0 in ORG and IP fruits, respectively). Of all the cultivars investigated, only ORG 'Golden Delicious' russeting could be considered as a commercially important negative fruit characteristic. Although russetted 'Golden Delicious' are not sold as fresh fruit in the U.S. marketplace, in Italy some retailers sell fully russetted 'Golden Delicious' apples.[12] A strong effect of cultivar on apple physicochemical and nutritional quality is widely recognized,[5,6,36] and it has been suggested that differences between apple cultivars have a far greater influence on fruit quality parameters than differences in production systems.[37]

Total Polyphenol Content, Low and High Molecular Weight Proanthocyanidins, Total Anthocyanins, and Antioxidant Capacity. The content of total polyphenols and different flavonoid groups, that is, low and high molecular weight proanthocyanidins and total anthocyanins, together with the total antioxidant capacity of ORG and IP fruit samples of the four cultivars investigated is presented in Table 2. There were nonsignificant differences in total polyphenols and total anthocyanin content between the ORG and IP management systems in three of the four cultivars investigated, all scab-resistant ('Liberty', 'Santana', 'Topaz'). Similarly, over a four-year study, Peck et al.[35] did not find any significant differences in total phenol content and antioxidant capacity between ORG and IP systems in scab-resistant 'Liberty' fruit. In another study, Valavandis et al.[21] concluded that ORG apples did not have either a significantly higher phenolic content or antioxidant capacity as compared to conventionally grown ones for the five cultivars investigated. In contrast, in a two year study on 'Florina', 'Topaz', 'Reinette de Champagne', and 'Crown Prince Rudolf' apples, on average, a higher total polyphenol content (not always significant) was found in the peel and pulp of ORG apples in comparison to IP apples.[16] Interestingly, the exception in our study was a scab-susceptible 'Golden Delicious', for which significantly higher total polyphenols and both low and high molecular weight proanthocyanidin contents were found in ORG 'Golden Delicious' fruit (Table 2). 'Golden Delicious' is not red; therefore, anthocyanins were not detected in 'Golden Delicious' fruit. Even if significant differences were found in phenolics content between ORG and

Table 3. Contents of Phenolics from Different Classes in Apples Grown Using Organic (ORG) and Integrated Production (IP) Systems

		mg/kg FW			
	production system	Golden Delicious	Liberty	Santana	Topaz
benzoic acid derivatives					
4-hydroxybenzoic acid	ORG[a]	0.011 a[b]	0.054 a	0.070 a	0.161 a
	IP	0.006 b	0.045 a	0.052 a	0.140 a
vanillin	ORG	0.131 a	0.153 a	0.184 a	0.279 a
	IP	0.092 a	0.104 a	0.243 a	0.264 a
vanillic acid	ORG	0.012 a	0.019 a	0.028 a	0.006 a
	IP	0.005 a	0.028 a	0.039 a	0.007 a
3,4-dihydroxybenzoic acid	ORG	1.462 a	1.109 b	1.760 a	0.726 a
	IP	0.942 a	2.542 a	2.412 a	0.146 a
phenylpropanoids					
neochlorogenic acid	ORG	0.182 a	1.071 a	0.165 a	0.076 a
	IP	0.110 b	1.195 a	0.178 a	0.085 a
cryptochlorogenic acid	ORG	2.545 a	19.117 a	2.633 a	0.082 a
	IP	2.637 a	23.394 a	2.168 a	0.085 a
chlorogenic acid	ORG	73.051 a	78.065 a	59.252 a	41.249 a
	IP	34.714 b	76.051 a	73.368 a	47.620 a
dihydrochalcone					
phloridzin	ORG	20.353 a	9.119 a	12.356 a	6.443 a
	IP	8.638 b	8.197 a	7.809 b	6.245 a
flavan-3-ols					
catechin	ORG	3.721 a	25.761 a	0.471 a	7.930 b
	IP	3.918 a	25.012 a	0.535 a	9.568 a
epicatechin	ORG	32.071 a	29.106 a	5.597 b	51.241 b
	IP	29.954 a	31.948 a	7.041 a	58.448 a
procyanidin B1	ORG	15.275 a	42.663 a	1.181 a	22.749 b
	IP	12.385 a	45.360 a	0.996 a	29.251 a
procyanidin B2+B4	ORG	83.488 a	40.424 a	8.412 a	95.199 a
	IP	62.901 b	46.436 a	7.938 a	104.063 a
flavonols					
quercetin-3-O-rhamnoside	ORG	28.999 a	27.321 a	12.866 a	47.185 a
	IP	23.418 a	22.525 a	12.633 a	50.911 a
quercetin-3-O-glucoside	ORG	10.904 a	13.225 a	17.483 a	7.104 a
	IP	5.818 b	12.111 a	14.230 a	7.152 a
quercetin-3-O-galactoside	ORG	63.364 a	37.220 a	21.594 a	39.249 a
	IP	30.300 b	26.041 a	16.467 a	36.668 a
isorhamnetin-3-O-glucoside	ORG	0.041 a	3.874 a	4.010 a	0.024 a
	IP	0.024 a	2.811 a	4.971 a	0.008 a
kaempferol-3-O-rutinoside	ORG	0.039 a	0.147 a	0.104 a	0.058 a
	IP	0.012 b	0.074 a	0.078 a	0.052 a
rutin	ORG	3.997 a	6.380 a	4.286 a	3.055 a
	IP	1.160 b	3.483 a	2.891 a	2.745 a
isorhamnetin-3-rutinoside	ORG	0.012 a	1.484 a	1.285 a	0.013 a
	IP	0.007 a	0.753 a	1.256 a	0.006 b
summarized	ORG	339.658	336.312	153.737	322.829
summarized	IP	217.041	328.110	155.305	353.464

[a]Numbers of ORG and IP samples for each cultivar are shown in Tables 1 and 2. [b]Different letters indicate a significant difference between ORG/IP means in each cultivar with F test at $P \leq 0.05$.

IP 'Golden Delicious' fruits, there were no significant differences in antioxidant capacity between them. The reason for that was most probably the contribution of other phytochemicals such as vitamin C to the antioxidant capacity of apples.[7] It has to be considered that the Folin−Ciocalteu assay in this study aimed to evaluate total polyphenols in apples, not including polar compounds, such as vitamin C. On the other hand, the total antioxidant capacity assay aimed to determine total antioxidant capacity of fruit, including phenolics and other antioxidants. On another hand, ORG 'Santana' fruit

Table 4. Sensorial Parameters of Apples Grown Using Organic (ORG) and Integrated Production (IP) Systems

cultivar	triangle (% of correct answers)	production system	sweetness (1–9)	tartness (1–9)	crispness (1–9)	firmness (1–9)	juiciness (1–9)	overall flavor (1–9)	appearance (1–9)
Golden	50[a]	ORG[b]	6.4 a[c]	4.0 b	4.6 b	4.3 b	5.1 a	5.7 b	4.9
Delicious		IP	6.3 a	4.6 a	5.7 a	5.5 a	5.3 a	6.6 a	6.9
Liberty	37	ORG	5.8 a	4.7 a	4.9 b	4.8 b	5.4 b	5.6 b	6.5
		IP	5.7 a	5.0 a	6.0 a	5.6 a	6.2 a	6.4 a	7.6
Santana	47[a]	ORG	4.5 b	6.6 a	6.1 a	5.3 a	6.6 a	5.7 b	5.3
		IP	5.5 a	5.0 b	6.0 a	5.2 a	6.3 a	6.6 a	6.7
Topaz	58[a]	ORG	4.7 a	5.8 a	4.6 a	4.3 a	5.3 a	5.8 a	5.2
		IP	4.6 a	5.8 a	4.6 a	4.1 a	4.8 a	5.4 a	6.6

[a]Shows significant taste difference between ORG/IP samples tested using triangle test at $P \leq 0.05$. [b]Numbers of ORG and IP samples for each cultivar are shown in Tables 1 and 2. [c]Different letters indicate a significant difference between ORG/IP means in each cultivar with F test at $P \leq 0.05$.

had a significantly higher antioxidant capacity, together with significantly higher low and high molecular weight proanthocyanidin content, but no significant differences between the ORG and IP systems were found in terms of total polyphenol content (Table 2). With regard to human health, 'Santana' is known as a scab-resistant cultivar suitable for more environmentally friendly farming, at the same time being a hypoallergenic cultivar. 'Santana' apples cause significantly fewer allergic symptoms in apple-allergic individuals than 'Golden Delicious' or 'Topaz'.[26] Interestingly, in comparison to the other three cultivars investigated, 'Santana' apples had the lowest total phenol content (on average, 592 and 567 mg/kg in ORG and IP fruits, respectively), as well the lowest low and high molecular weight proanthocyanidins among all investigated cultivars. On average, the highest content of all phenol groups was found in the ORG 'Golden Delicious' apples, followed by IP and ORG 'Liberty' fruits (Table 2). The results obtained are consistent with those of Vrhovsek et al.,[5] who observed on average higher total phenol content in "old" cultivars, 'Renetta', 'Red Delicious', 'Granny Smith', 'Morgenduft', and 'Golden Delicious' (2119, 1311, 1210, 1058, and 863 mg/kg FW of (+)-catechin, respectively), as compared to "new" cultivars, 'Royal Gala', 'Braeburn', and 'Fuji' (839, 754, and 662 mg/kg, respectively). On the other hand, in the study by Wojdyło et al.[6] it was demonstrated that among 67 apple cultivars, "new" apples had on average the same or higher phenol content than the "old" ones.

Determination of Multiple Classes of Phenolics. A versatile targeted metabolomic UPLC-MS/MS method used in the study enabled rapid separation, detection, and quantitation of as many as 135 phenolic metabolites from multiple phenolic classes: benzoic acid derivatives, coumarins, phenylpropanoids, stilbenes, dihydrochalcones, isoflavones, flavones, flavanones, flavan-3-ols, and flavonols.[27] High molecular weight proanthocyanidins and anthocyanins, the latter requiring particular chromatographic conditions (i.e., low pH), were not included in the study, but were determined spectrophotometrically (see above). In apple aqueous 70% acetone extracts of ORG and IP fruits analyzed using this method, as many as 19 phenolics from different classes were detected at a concentration above the limit of quantitation (Table 3; Figure S1 in the Supporting Information). The method also enabled good separation of some isomeric forms, such as chlorogenic acids. Procyanidins

B2 and B4 were not separated well and were therefore quantified as a single compound (Figure S1). In the presented one-year experiment some metabolic fluctuation in phenolics in relation to agricultural practice was detected in all four cultivars investigated. However, considerable differences were found only in 'Golden Delicious' fruit. In 'Golden Delicious' fruit, 9 of 19 detected phenols were significantly higher in ORG than in IP fruits. These results are consistent with spectrophotometric analysis, in which total polyphenol content and low and high molecular weight proanthocyanidins were significantly higher in ORG 'Golden Delicious' fruits compared to IP (Table 2). ORG 'Golden Delicious' fruits had significantly higher levels of 4-hydroxybenzoic acid, neochlorogenic and chlorogenic acid, phloridzin, procyanidin B2+B4, quercetin-3-O-glucoside, quercetin-3-O-galactoside, kaempferol-3-O-rutinoside, and rutin (Table 3). It seems that the ORG agricultural system caused some kind of "up-regulation" of certain phenolic classes, especially benzoic acid derivatives, phenylpropanoids, and flavonols in scab-susceptible 'Golden Delicious' fruit. On the other hand, the fluctuations were far less significant in all of the scab-resistant cultivars investigated. ORG 'Santana' fruits had a significantly higher level of phloridzin and a significantly lower level of epicatechin. ORG 'Liberty' fruits had a significantly lower level of 3,4-dihydroxybenzoic acid, whereas ORG 'Topaz' fruits had significantly lower level of some flavan-3-ols, that is, catechin, epicatechin, procyanidin B1, and procyanidin B2+B4, the latter not significant. The results obtained were consistent with the spectrophotometrically determined lower content of low molecular weight proanthocyanidins in ORG 'Topaz' as compared to IP (Table 2). Considering the content of different phenolic classes for both the ORG and IP systems, all of the cultivars investigated had on average the highest content of flavan-3-ols (except 'Santana'), followed by phenylpropanoids and/or flavonols (Table 3). These results are consistent with previous results.[5,6] The summarized content of detected phenolic classes was lowest in the ORG and IP 'Santana' cultivar (153.7 and 155.3 mg/kg, respectively) and surprisingly highest in IP 'Topaz' (353.5 mg/kg), followed by ORG 'Golden Delicious' (339.7 mg/kg) and ORG and IP 'Liberty' (336.3 and 328.1 mg/kg, respectively) (Table 3). Again, in the case of the summarized contents, the strongest impact of the ORG system on higher levels of phenolics was found in the scab-susceptible

'Golden Delicious' cultivar but not in the other three scab-resistant cultivars.

Sensorial Quality. The results of sensorial tests are presented in Table 4. Using the triangle test, panelists were able to significantly distinguish between the taste of apples grown using ORG and IP systems in the case of 'Santana', 'Golden Delicious', and 'Topaz'. In the case of 'Liberty' 37% correct assessments were on the border of significance (but not significant at $P \leq 0.05$ level) when a one-in-three chance random selection was performed. Similarly, Peck et al.[35] reported that consumer panelists were able to distinguish between ORG and IP fruits of 'Liberty' using the triangle test, but in double-blind hedonic/intensity tests they did not consistently rate one treatment higher than the other. The hedonic/intensity test in terms of sweetness, tartness, crispness, firmness, juiciness, and overall flavor in our study also showed some inconsistent differences between ORG and IP fruit of different cultivars (Table 4). ORG-grown 'Golden Delicious' apples were significantly less tart, less crisp, and less firm and rated a significantly lower score for overall flavor. ORG-grown 'Liberty' apples were also less crisp, less firm, and less juicy and rated a significantly lower score for overall flavor. ORG-grown 'Santana' apples were perceived as significantly less sweet nad more tart and rated a lower score for overall flavor. In the case of 'Topaz', the differences between the two management systems in the hedonic/intensity test were not significant. Panelists scored the overall appearance of ORG apples at least 1 point (of 9) lower than fruit from the IP system. Sensorial evaluation indicated both significantly better flavor (except for 'Topaz') and appearance for IP-produced apples. The better overall appearance of IP-cultivated apples could be explained by a more efficient plant protection spraying program. Thus, IP apples were bigger (although not significantly at $P \leq 0.05$), had no apple scab, and had less russet or other skin malformations.

In summary, consumers purchase ORG food because they believe it offers better sensorial quality, such as freshness and taste, and a higher nutritional value.[38] However, the complexity of fruit quality parameters, including a range of sensory parameters, the nutritional value (in terms of phenolics, vitamins, fibers content), the presence/absence of pesticide residues, benefits to the environment and society, etc., often make it difficult to reach general conclusions. One-year study results have shown that in three of the four cultivars consumers preferred both the overall flavor and appearance of IP fruit. With regard to the nutritional value in terms of phenolics, there was a nonsignificant increase in total polyphenols content in ORG as compared to IP apples in three of the four cultivars investigated in 2010, all scab-resistant, that is, 'Liberty', 'Santana', and 'Topaz'. The exception was a scab-susceptible 'Golden Delicious', in which significantly higher content of total polyphenols and proanthocyanidins was found in ORG fruit. Targeted metabolomic profiling of different phenolic classes in fruits from both systems confirmed that the ORG system significantly affected the up-regulation of a number of phenolics in 'Golden Delicious' apples. The fluctuations were far less significant in all of the scab-resistant varieties investigated, that is, 'Liberty', 'Santana', and 'Topaz'. It could be surmised that scab resistance is one of the factors which differentially affect multiple classes of phenolic biosynthesis in relation to the agricultural system. The results are based on a one-year study. However, multiyear research is needed for better understanding of how agricultural systems influence some sensory attributes and gene expression for phenolics biosynthesis between resistant and susceptible apple varieties. The multifunctionality of phenolics in plants, however, often complicates interpretation of experimental results. Carefully designed and holistic studies combining 'omics' technologies, that is, genomics, transcriptomics, proteomics, and metabolomics, are urgently needed for a deeper understanding of how agricultural practice and other factors differentially affect apple cultivars in terms of their resistance and their sensorial and nutritional quality.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

Figures S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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