

Article

Analysis of Apple Fruit (*Malus × domestica* Borkh.) Quality Attributes Obtained from Organic and Integrated Production Systems

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Abstract: The aim of this study was to compare total phenolic content (TPC), radical-scavenging activity (RSA), total anthocyanin content (TAC), sugar and polyphenolic profiles of two apple cultivars (‘Discovery’ and ‘Red Aroma Orelin’) from organic and integrated production systems in climatic conditions of Western Norway. Sixteen sugars and four sugar alcohols and 19 polyphenols were found in the peel, but less polyphenols were detected in the pulp. The peel of both apples and in both production systems had significantly higher TPC and RSA than the pulp. The peel from integrated apples had higher TPC than the peel from organic apples, while organic apples had higher TAC than the integrated. Sucrose and glucose levels were higher in organic apples; fructose was cultivar dependent while minor sugars were higher in integrated fruits. The most abundant polyphenolic compound in the peel of the tested cultivars was quercetin 3-O-galactoside, while chlorogenic acid was most abundant in the pulp. Regarding polyphenols, phloretin, phloridzin, protocatechuic acid, baicalein and naringenin were higher in organic apple, while quercetin 3-O-galactoside, kaempferol 3-O-glucoside, chlorogenic acid and syringic acid was higher in integrated fruits. In conclusion, organic ‘Discovery’ and integrated ‘Red Aroma Orelin’ had higher bioavailability of health related compounds from the peel and the pulp.

Keywords: sugar profile; phenolics; total phenolic content; radical-scavenging activity; total anthocyanin content; Norway

1. Introduction

Currently, three different agricultural production systems are dominant worldwide. ‘Conventional’ (industrial, large scale) agriculture production, which rose from Norman Borlaug’s “Green Revolution”, is highly mechanized and organized, with synthetic fertilizers, pesticides, and lately with genetically modified organisms (GMO). The second type is an opponent system, called ‘Organic’ production (regulated by Council Regulation (EC) 834/2007), which is leaning on natural inputs, diversification and greater labor and is gaining more popularity (together with biodynamics as a sub variant). The last one is ‘Integrated’ (directed by directive 2009/128/EC), which is the best management practice and stands between conventional and organic production [1].

Globally, 1.5% of farmland is organic with more than 2.8 million producers [2]. In some countries the share is much higher, while some are or aspire to be 100% organic in

the future. The global market for organic food reached 106 billion Euros, with USA as the leading consumer country [2]. The main reason why people are buying organic food is the supposition that organic products have no or a significantly lower amount of synthetic pesticide's residues when compared to conventional products [3]. Earlier, organic products could be bought only at farmers' markets and local food stores, but now they are sold in every mainstream supermarket [4]. Besides banana, apple is a fruit species with the most area under organic management, and both have expanded rapidly during the last decade [5]. Organic apple world production is ~114,000 ha (2% organic land), where China is leading (30,000 ha), followed by the USA (11,000 ha) [2]. Integrated production is a method that uses controlled amounts of synthetic pesticides. However, consumers are developing a preference for organic production due to the environmentally friendly alternatives which encompass the sustainable use of energy and natural resources. Besides, up keeping of biodiversity, the preservation of ecosystems, the increment of soil fertility, animal welfare and the decreased pollution of water, soil, and air are also some of the advantages of organic production [6]. Since some apple cultivars can be sprayed from 15 up to 22 times, with numerous different pesticides, consumers have started to be aware of the health risks for themselves and for farm workers who are exposed to pesticides, not to mention the accelerating production costs [7]. Besides that, lower levels of toxic metabolites (heavy metals, synthetic fertilizer and pesticide residues) and lower exposure to antibiotic-resistant bacteria are pushing organic production forward [8].

Apple (*Malus × domestica* Borkh.) is a temperate zone fruit, but on a global level it is economically and culturally one of the most important fruit species [9]. Regarding fruit production worldwide, apples (86 million tons) are ranked second after bananas (120 million tons), but before grapes (78 million tons) and oranges (75.5 million tons). In 2020, China was the leading producer of apples worldwide, with ~40.5 million tons (47%) [10]. Pleasant aroma and taste, high yields, low prices, good transportability, less fruit deteriorating and long storage mean that apples are eaten year-round, with an average worldwide daily consumption of ~200 g per capita [11]. Furthermore, nutritional qualities including high levels of carbohydrates, organic acids, minerals, vitamins, dietary fibers, pectin, chlorophyll, and carotenoids are making this temperate fruit species highly appreciated by consumers [12,13]. Other phytochemicals include phenolics such as flavanols, flavan-3-ols, flavanones, phenolic acids, anthocyanins, triterpenoids, and others [14,15]. The quantity of phytochemicals in apple fruit depend on cultivar, rootstock, cultural and growth conditions, plant nutrition, storage and processing together with biotic and biotic stresses, especially during the maturation of the fruits [16]. Apples are showing very high antioxidant activity, thus preventing many chronic diseases [17]. Diets rich in apples and apple products are linked with reduced risks of some cancers, cardiovascular disease, asthma, Alzheimer's disease, obesity and diabetes [18]. Its consumption improves bone and gastrointestinal health and pulmonary function [19]. *Malus × domestica* fruits have both dessert and culinary uses, thus in most cases they are consumed fresh as snacks, or used for making juice, concentrate, marmalade, jam, compotes, tea, wine, dried fruits and cider [20]. Apple pomace is used for pectin recovery, the bioproduction of citric acid, in herbal tea production and as feed formulations for racing pigeons [21]. Seeds, as waste, which are left over after apple processing, have up to 27% of oils rich in fatty acids (>95% of unsaturated fatty acids), carotenoids and tocopherols [22,23].

Organic production in Norway takes up more than 45,000 ha (~2000 producers), which is 4.6% of the country's total agricultural land. Out of this, apple production is done on 164 ha (11% of organic land) [24]. Apples (mostly the cultivars 'Discovery' and 'Red Aroma Ore lind') are grown in Southern, Eastern and Western Norway where the climate and growing conditions for apples are the most suitable [25]. The demand for organic Norwegian produced fruits is large, but mostly imported organic fruit from other countries are sold.

In the last 10–15 years, many scientific studies have been performed in order to compare integrated/conventional and organic apple production and fruit quality mostly regarding physical, chemical, and sensorial traits [7,12,26–29]. Back in 1997, Woese et al. [30] showed that no major differences could be observed between organically and conventionally produced apples with respect to vitamins (B1, B2, C), carbohydrates, organic acids, proteins and free amino acids. Contrary to this, Peck et al. [7] and Holb et al. [28] reported better physicochemical quality (skin blush, soluble solids content, organic acids, flesh firmness, minerals and fiber) of organic apples with 10–15% higher antioxidant activity. Regarding phenolics, Vanzo et al. [31] and Średnicka-Tober et al. [29] found higher levels of 4-hydroxybenzoic acid, neo chlorogenic and chlorogenic acid, phloridzin, procyanidin B2 + B4, kaempferol-3-O-rutinoside, rutin and anthocyanins in organic fruits compared to conventional ones, while Lamperi et al. [32], Valavanidis et al. [33] and Santarelli et al. [34] demonstrated that organic production methods did not significantly contribute to the polyphenol content. Adamczyk et al. [35] found that organic apples had better taste, while Róth et al. [27] proved that trained sensory panelists could not differentiate between organic and conventional apples in terms of aroma and volatiles. Many researchers reported that divergent results regarding fruit quality from organic and conventional/integrated production could be a reflection of distinctive seasons, sites, cultivars, orchard management and nutritional supply. Due to the fact that the two apple production systems have never been confronted in a comprehensive way in Western Norway, the aim of this study was to compare the sugar profile of the whole fruit and polyphenolic profiles of the peel and pulp of fruits from cultivars ‘Discovery’ and ‘Red Aroma Ore lind’ grown in organic and integrated production systems. Furthermore, another goal was to determine the magnitude for some key nutrients between examined cultivars and to recommend which cultivar is for which production system in this, or similar, agro-climatic conditions.

2. Materials and Methods

2.1. Plant Material and Managements

This study was set up in 2018, in two apple orchards in the Hardanger region of western Norway. Integrated pest management was applied in one orchard and organic production in the second. The organic apple orchard was located at the experimental farm of NIBIO Ullensvang (60.318655, 6.652948) and the conventional orchard at a private grower in Ullensvang (60.211060, 6.604015). The locations of these orchards were typical for the region, the main fruit production area in Norway, and both represented the same climate zone. The soil in the area is mainly moraines that were left by the glaciers after the last glaciation 10,000 years ago. It has high contents of stones, but is a splendid medium for fruit growing, being rich in minerals and humus and with good water capacity. The soil in both orchards was a sandy-loam with approximately 5% organic matter, being very uniform in morphological and physical characteristics (color and structure). Soil composition, organic matter content, CEC-values (Cation exchange capacity), pH, nutrient concentrations and plant-available amounts of nutrients are monitored in this area [36].

In both orchards studied, the cultivars ‘Discovery’ and ‘Red Aroma Ore lind’ were grafted on M9 rootstocks spaced 1×3.5 m apart at the organic site and 1.5×4 m apart at the integrated site. Two-year old knip-trees were planted May 2015 at the organic side and the integrated trees were two years older. Both cultivars have scab tolerance and are the main commercial cultivars for both integrated and organic production methods. All trees were trained as spindle trees and pruned to a maximum height of about 2.5–3 m. The selected trees were homogeneous in terms of flower set, vigor, and health status in both orchards. The organic site was officially certified on 30 April 2018 by the Norwegian control body Debio, according to the Norwegian ‘Regulations on the Production and Labelling of Organic Agricultural Product’.

On the organic side, the weeds under the trees were removed by frequently mowing and using a rotator tiller and on the integrated side a 1m wide herbicide strip using glyphosat (trade name Roundup with 360 g/L of glyphosat, Monsanto Crop Sciences) (May 8, BBCH 56) was used, which was maintained each season together with frequent mowing grass in the interrows. In the organic orchard, trees were treated against the apple scab using four applications with sulphur (trade name Thiovit Jet with 80% sulphur as an active ingredient, Syngenta, Basel, Switzerland) during the season. The trees were fertilized with 200 kg/ha organic hen manure (pellets), 8% N, 4% P and 5% K in percentage of dry matter. In the integrated orchard, four applications against major pests were applied during the season, one time against insects (tiaklopid, 480 g/kg active ingredient, trade name Calypso SC 480, Bayer Crop Science, Leverkusen, Germany), two times against apple scab (diatianon, 700 g/kg active ingredient, trade name Delan WG, BASF, Ludwigshafen, Germany) and once against storage diseases (tiofanatmethyl, trade name Delan WG, Bayer Crop Science, Leverkusen, Germany). Each spring, 300 kg/ha Yara-Mila® FULLGJØDSEL® 12-4-18 micro was applied and in mid-June 200 kg/ha Yara-Liva® Kalksalpeter 16-0-0) was applied. In all fields, drip irrigation was installed with one drip line along the tree rows having 0.5 mm drip distance. The trees were regularly irrigated when water deficits occurred based on evaporation and precipitation and on average 2–3 mm was given daily in this relatively cool climate. All trees received the same amount of fertilizers based on soil analysis. Hand thinning was carried out at both locations at the end of June in order to achieve optimum crop loads of good fruit quality (15 cm apart between fruitlets).

2.2. Climate Conditions, Flowering and Harvesting Time

Fruit production in Norway is located in the Southern part of the country, which has the most favorable climate, with lakes in the Eastern part and fjord areas in the Western part adjacent to it. The fjord areas in Western Norway have a maritime climate, with relatively cool summers and mild winters. Weather fronts are usually coming from south-west from the North Sea and the Atlantic Ocean. It is rare that there are problems with frost damage to the fruit trees, either during the winter or during blossom time. The snow-covered mountains provide protection from high amounts of rain from the west. On the other hand, due to relatively cool summers, the climate is the main limiting factor behind a relatively short and cool growing season, which limits both the species and the cultivars to be grown. The climate in Ullensvang (western Norway) was a bit warmer during the 2018 season, relatively dry at the beginning of the season and very wet during the harvest periods during the fall. The average annual air temperature during the year was 8.4 °C with the average during the growing period, from May to October, being 14.1 °C. Total rainfall was 1534 mm, with 1030 mm in the growing season. In previous years, average figures for the year was 7.6 °C and 1705 mm and for the growing season 12.3 °C and 638 mm.

Full bloom for ‘Discovery’ (BBCH 65) was May 16 and for ‘Red Aroma Orelind’ it was May 21. The cultivar ‘Discovery’ was harvested (BBCH 89) in mid-September at both locations, and ‘Red Aroma Orelind’ was harvested one month later at the same time based on predicted harvest criteria for the cultivars. Each apple cultivar in each production system was represented by 30 trees (3 repetitions × 10 trees). Fruit quality characteristics and chemical analysis were undertaken on samples of 20 collected fruits per cultivar/per production system/per repetition. Sample fruits were picked randomly from all trees within one repetition, from all four main directions around the tree canopy, and from the upper, middle and lower third of the crown.

The maturity levels were the same for both cultivars. Right after harvests the starch contents were measured calorimetrically after staining the flesh of a halved apple with a mixture of 1% iodine and 4% potassium-iodide and indexing the surface colour on a scale of 1 (dark blue color = high starch content) to 9 (no blue color = no starch). Firmness was measured using a FTA penetrometer (www.aceindustrial.co.uk, accessed on 1 September

2018) equipped with an 11-mm plunger. No starch was left when analysing and the fruit firmness was about 6 kg per cm². Immediately after these tests the apple fruits were analyzed.

2.3. Reagents and Standards

Acetonitrile and formic acid (both MS grade), methanol (HPLC grade), Folin-Ciocalteu reagent, sodium carbonate, sodium hydroxide, hydrogen peroxide, and hydrochloric and nitric acid were purchased from Merck (Darmstadt, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Sigma Aldrich (Steinheim am Albuch, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and sodium acetate were purchased from Fluka AG (Buch, Switzerland). Ultra-pure water (ThermoFisher TKA MicroPure water purification system, 0.055 µS/cm) was used to prepare the standard solutions and blanks. Syringe filters (13 mm, nylon membrane 0.45 µm) were purchased from Supelco (Bellefonte, PA, USA).

Polyphenolic standards were purchased from Fluka AG (Buch, Switzerland). The standards of sugars and polyols were purchased from Sigma-Aldrich (Steinheim am Albuch, Germany), whereas sodium acetate trihydrate and sodium hydroxide were obtained from Merck (Darmstadt, Germany). All aqueous solutions were prepared using Ultrapure TKA water.

2.4. Sample Preparation

The extraction of phenolics from the apple peel and pulp was done by the method previously described by Pantelić et al. [37]. A representative sample of 20 fruits (per replication/per cultivar/per production system) was divided into two parts. Ten fruits were ground together with the peel and 50 g of that mass was taken for the analysis of the sugar profile. The other ten fruits were peeled, and 2 g was taken from the peels, and 5 g from all fruit's mesocarps for the analysis of polyphenolic profiles and tests. The peel (2 g) and pulp (5 g) were mixed with 20 mL methanol containing 0.1% HCl and stirred for 1 h on a magnetic agitator. Extractions were repeated three times and all three fractions were collected, combined, and evaporated to dryness by rotary evaporator IKA RV8 (IKA®—Werke GmbH & Co. KG, Breisgau-Hochschwarzwald, Germany) under reduced pressure at 40 °C. The residue after evaporation was dissolved in 10 mL of ultrapure water and these solutions were used for further analysis. The extracts were filtered through a 0.45 µm PTFE membrane filter before analysis.

2.5. Preparation of Standard Solutions

A 1000 mg/L stock solution of a mixture of all phenolic standards was prepared in methanol. Dilution of the stock solution with mobile phase yielded the working solution of concentrations 0.025, 0.050, 0.100, 0.250, 0.500, 0.750, and 1.000 mg/L, respectively.

The evaluation of the carbohydrate content of the samples was obtained from the calibration curves of the pure compounds. The calibration was performed with standard solutions of sugars and sugar alcohols dissolved in ultrapure water. Under these chromatographic conditions, the last compound was detected after approximately 25 min, and the analysis was ended at 30 min.

2.6. UHPLC–DAD MS/MS Analysis of Polyphenolic Compounds

The determination of the phenolic compounds in the apple peel and pulp samples were performed using a Dionex Ultimate 3000 UHPLC system equipped with a diode array detector (DAD) that was connected to TSQ Quantum Access Max triple-quadrupole mass spectrometer (ThermoFisher Scientific, Basel, Switzerland). The elution was performed at 40 °C on a Synchronis C18 column. A TSQ Quantum Access Max triple-quadrupole mass spectrometer equipped with a heated electrospray ionization (HESI) source was operated in negative ionization mode. The mobile phase consisted of

ultra-pure water + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B), which were applied in the following gradient elution: 5% B in the first 1.0 min, 1.0–16.0 min 5–95% B, 16.0–16.2 min from 95% to 5% B, and 5% B until the 20th min. The flow rate was set to 0.3 mL/min. The detail setting of the mass spectrometry detector was previously described in Gašić et al. [38]. Xcalibur software (version 2.2) was used for instrument control. The phenolics were identified by direct comparison with commercial standards. The total amounts of each compound were evaluated by calculation of the peak areas and are expressed as mg/kg.

2.7. Total Phenolic Content (TPC)

The total phenolic content was determined using the Folin–Ciocalteu method [39] following the procedure described in [40]. Briefly, 0.5 mL of the apple peel/pulp extracts and 0.5 mL ultrapure water were mixed with 2.0 mL of 10% Folin–Ciocalteu reagent. After 5 min, 2.5 mL of 7.5% sodium carbonate was added. The mixture was left to stand for 2 h and the absorbance was measured at 765 nm. Gallic acid (20–100 mg·L⁻¹) was used as a standard and the results were expressed as gram gallic acid equivalent (GAE) per kg of fresh weight (FW). TPC amounts were presented as mean values of three measurements ± SD.

2.8. Radical-Scavenging Activity (RSA)

Radical scavenging activity was determined using DPPH radical solution by a literature method [37]. An aliquot of 0.1 mL of extracts was mixed with 4 mL of methanol solution of DPPH (71 µM). The mixture was left to stand for 60 min in the dark and the absorbance was measured at 515 nm. Trolox was used as standard and the calibration curve was displayed as a function of the percentage of inhibition of DPPH radical. The results were expressed as milimoles of Trolox equivalents (mmol TE) per kg of fresh weight and were presented as mean values of three measurements ± SD.

2.9. Total Anthocyanin Content (TAC)

The total anthocyanin content was determined by the pH-differential method [40] and results were presented as gram cyanidin-3-glucoside (cyn-3-glu) per kg of fresh weight. Apple peel extracts were diluted with buffers of pH 1.0 (hydrochloric acid–potassium chloride, 0.025 M) and pH 4.5 (acetic acid–sodium acetate, 0.4 M). Absorbencies of the extracts were measured at 510 and 700 nm against blank. All of the results were presented as mean values of three measurements ± SD.

2.10. HPAEC/PAD Analysis of Sugars and Sugar Alcohols

Fifty g of the representative fresh apple sample were minced and mixed with 100 mL of the ultra-pure water and homogenized in a shaker for 15 min. The mixture was centrifuged at 7000 rpm for 20 min. The supernatant was filtered through 22 µm syringe filters and kept in a freezer until analysis.

The carbohydrate analysis was performed on a DIONEX ICS 3000 equipped with a quaternary pump and a pulsed amperometric detector (PAD) with a glass electrode as referent and gold as a working electrode. All of the separations were performed on a CarboPac PA 100 column (4 × 250 mm) (Dionex, Sunnyvale, CA, USA). The flow rate was 0.7 mL/min. At the beginning of the analysis the mobile consisted of 85% water and 15% 300 mM sodium hydroxide. From 5 to 6 min the composition was changed to 83% water, 15% 300 mM sodium hydroxide and 2% 500 mM sodium acetate. The next change was from 12–13 min where the water content decreased to 81% and the content of the 500 mM of sodium acetate was increased to 4%. The final concentration was obtained between 20–21 min and consisted of 60% water, 20% 300 mM sodium hydroxide and 20% 500 mM sodium acetate. Before every analysis the system was equilibrated to starting conditions for 30 min. All of the separations were performed at 30 °C.

The total sweetness index (TSI) was calculated in order to determine the sweetness perception of fruits with the following equation:

$$\text{TSI} = (1.00 \times [\text{sucrose}]) + (0.76 \times [\text{glucose}]) + (1.50 \times [\text{fructose}]).$$

2.11. Statistics

Data from all the measurements were expressed as the mean of three replicates. A Tukey's test was used to detect the significance of the differences ($p \leq 0.05$) among mean values. Statistical analyses were performed using the NCSS program (<https://www.ncss.com/>, accessed on January 29, 2004). A principal component analysis was done in order to summarize the result of the polyphenols, spectrophotometric tests and sugar contents in investigated apple samples. PCA was carried out using the PLS_Tool Box software package for MATLAB (Version 7.12.0), Budapest, Hungary as described in Fotirić Akšić et al. [6].

3. Results and Discussions

3.1. Determination of TPC, RSA, and TAC

TPC values obtained for the peel were higher in the cultivar 'Red Aroma Oreind' (16.45 g GAE/kg FW in organic fruits and 22.89 g GAE/kg FW in integrated fruits) compared to 'Discovery' (11.33 g GAE/kg FW in organic fruits and 17.75 g GAE/kg FW in integrated fruits). In the pulp, the contents of total phenols were higher in the cultivar 'Discovery' (Table 1). However, peel in 'Discovery' were 11-fold higher TPC than in the pulp, while in 'Red Aroma Oreind' it was ~20-fold higher (Table 1). This is probably due to the fact that apple peels contained additional flavonoids, such as quercetin glycosides [41]. Therefore, although the peel only comprised 7% of the apple weight; it contributes up to 45% of the total polyphenols [42].

In this study, in all investigated samples, amounts of total phenolics were higher in samples grown in integrated production systems, which aligns with the findings of Valavanidis et al. [33], but does not correspond to the results of Santarelli et al. [34] and Vanzo et al. [31], who found no significant differences in TPC between production practices. According to Mikulič Petrovšek et al. [43], the higher TPC in organic production is mostly connected to stress caused by pests (especially scab infection). However, in west Norway *Venturia inaequalis* is not a big problem, and thus the level of stress is lower. This suggests that it is hard to understand what triggers and makes the difference in the secondary metabolites accumulate in apple fruit. Apple pulp samples contained similar amounts of total phenolics, while apple peel samples were notably higher compared with the results of phenolic content data from other publications [44,45].

The apple peel of 'Discovery' from organic production had much higher RSA than the peel from fruits produced in the integrated system; while it was opposite in 'Red Aroma Oreind'. It could be underlined that the antioxidant capacity in the apple fruit is genetically dependent, as previously determined by Kalinowska et al. [46]. Contrary to this, Lamperi et al. [32] found that in four apple cultivars ('Annurca', 'Golden Delicious', 'Red Chief' and 'Stayman Neepling'), the peels of organic fruits showed higher radical scavenging properties than corresponding ones from integrated production. On the top, Yuri et al. [47] found no significant influence of the cultivation management on RSA in 'Gala', 'Granny Smith', or 'Fuji'.

On the contrary, TAC values determined in apple peels were higher in organically grown apples. The obtained amounts of TAC are in accordance with the literature data [41,45].

Table 1. Total phenolic content, radical-scavenging activity and total anthocyanin content of apple peel and pulp of two apple cultivars organically and integrally grown.

Sample	Peel		TAC ***	Pulp	
	TPC *	RSA **		TPC *	RSA **
Discovery (organic p.)	11.33 ± 0.21 ^d	120.88 ± 0.00 ^b	0.54 ± 0.02 ^a	0.95 ± 0.02 ^c	25.58 ± 0.42 ^c
Discovery (integrated p.)	17.75 ± 0.05 ^b	103.21 ± 1.14 ^d	0.25 ± 0.00 ^c	1.60 ± 0.01 ^a	32.04 ± 0.53 ^a
Red Aroma Ore lind (organic p.)	16.45 ± 0.18 ^c	118.21 ± 1.89 ^c	0.29 ± 0.01 ^b	0.91 ± 0.02 ^c	28.18 ± 0.11 ^b
Red Aroma Ore lind (integrated p.)	22.89 ± 0.51 ^a	136.21 ± 0.94 ^a	0.18 ± 0.00 ^d	1.05 ± 0.00 ^b	28.92 ± 0.11 ^b

* TPC values are expressed as g GAE/kg FW. ** RSA is expressed as mmol TE/kg FW. *** TAC is expressed as g cyn-3-glu/kg FW. Different letters within the same column indicate statistically significant difference at $p < 0.05$ by Tukey's test.

3.2. Sugars and Sugar Alcohols Profiles

Sugars are primary products of photosynthesis, providing energy and carbon building for all biochemical processes, but they also determine fruit sweetness at harvest [48]. The ripening process, the plant's age, soil characteristics, microclimatic conditions, agrotechnical measurements, and the cultivar all affect the quantitative variations of sugars within the fruit and can be altered under the influence of biotic and abiotic stresses [49,50].

A total of 16 sugars and 4 sugar alcohols were quantified in the investigated apple samples (Table 2). Based on total sugar contents, investigated apple samples could be ranked as follows: integrated grown 'Red Aroma Ore lind' > organically grown 'Discovery' > organically grown 'Red Aroma Ore lind' > integrated grown 'Discovery'. According to Jakopič et al. [51], apples from integrated management practices contained higher levels of total sugars than organically produced apples. The most abundant sugar in all tested samples was fructose, followed by glucose and sucrose, which on average amounted to the 29.3%, 22.9%, and 7.7%, of all sugars detected, respectively. Regarding those three most abundant sugars, their content in the examined samples differed significantly. The higher fructose content was found in the integrated grown cultivar 'Red Aroma Ore lind' compared to the organic, and in organic 'Discovery' compared to integrated. This is very important to underline because fructose is recommended as a sweetener for diabetic patients, because it is a potent stimulator of lipogenesis which has negative effects in diabetes mellitus and in obesity [52]. The highest glucose (40.833 mg/g) and sucrose (17.560 mg/g) amounts were found in the organic apple 'Discovery'. The levels of the majority of minor sugars (trehalose, turanose, raffinose, isomaltotriose, maltose, galactose, ribose, galactitol, mannitol and xylose) were statistically higher in both integrated apple fruits compared to the organic ones.

On the contrary, there were no statistically significant differences for the average contents of glucose, fructose and sucrose obtained for different growing conditions. This is consistent with the findings of Ján and Davide [26] but different from the results of Bertazza et al. [53], who detected higher contents of monosaccharides in organic apple fruits and Kouřimská et al. [54], who detected higher levels of fructose and glucose in the organic apple cultivars 'Idared', 'Melrose', 'Šampion' and 'Zvonkové' and in integrated 'Ontario', 'Topaz' and 'Florina' compared to its counterparts. Those discrepancies are probably due to the different cultivars studied and the completely divergent climatic conditions.

The cultivar 'Discovery' had a significantly higher total sweetness index in organic production compared to the integrated cultivar, due to the higher level of all three 'big' sugars, while no discrepancies could be observed in 'Red Aroma Ore lind'. According to Aprea et al. [55] the sorbitol content correlates with the perceived sweetness better than

any other single sugar or total sugar content, which means that in the case of panelist organic ‘Discovery’ and integrated ‘Red Aroma Orelind’ would taste sweeter.

Table 2. Amounts of individual sugars and sugar alcohols (mg/g) in investigated apple cultivars organically and integrally grown.

Sugars	‘Discovery’		‘Red Aroma Orelind’		Cultivars		Production Systems	
	Integrated	Organic	Integrated	Organic	‘Red Aroma O.’	‘Discovery’	Integrated	Organic
Sorbitol	0.235 c	0.257 b	0.278 a,*	0.227 d	0.253 a	0.246 a	0.257 a	0.242 a
Trehalose	0.505 a	0.415 c	0.432 b	0.337 d	0.385 a	0.460 a	0.469 a	0.376 b
Arabinose	0.301 c	0.513 a	0.321 b	0.241 d	0.281 a	0.407 a	0.311 a	0.377 a
Glucose	37.410 b	40.833 a	30.586 d	31.918 c	31.252 b	39.122 a	33.998 b	36.376 a
Sucrose	16.078 b	17.560 a	12.968 d	14.887 c	13.928 b	16.819 a	14.523 a	16.224 a
Fructose	45.862 d	46.780 c	55.926 a	52.914 b	54.92 a	46.321 b	50.894 a	50.347 a
Isomaltose	1.296 b	1.214 c	1.363 a	1.049 d	1.206 a	1.255 a	1.330 a	1.132 b
Melezitose	0.063 c	0.073 b	0.127 a	0.056 d	0.092 a	0.068 a	0.096 a	0.064 a
Gentiobiose	0.019 c	0.005 d	0.096 a	0.061 b	0.078 a	0.012 b	0.014 a	0.005 a
Turanose	0.184 b	0.080 d	0.434 a	0.152 c	0.292 a	0.132 a	0.309 a	0.115 b
Raffinose	0.581 a	0.281 b	0.162 c	0.104 d	0.133 b	0.431 a	0.371 a	0.193 a
Isomaltotriose	0.033 c	0.027 d	0.264 a	0.104 b	0.184 a	0.030 b	0.149 a	0.065 a
Maltose	2.372 a	1.330 c	1.660 b	0.987 d	1.324 a	1.851 a	2.016 a	1.158 b
Panose	0.015 b	0.014 b	0.555 a	0.016 b	0.286 a	0.014 b	0.285 a	0.015 a
Maltotriose	0.008 c	0.007 c	0.521 a	0.020 b	0.270 a	0.007 b	0.264 a	0.013 a
Galactose	0.907 a	0.661 c	0.731 b	0.607 d	0.669 a	0.784 a	0.819 a	0.634 b
Galactitol	1.041 a	0.882 b	0.884 b	0.688 c	0.786 a	0.961 a	0.962 a	0.785 b
Ribose	0.411 b	0.337 c	0.506 a	0.296 d	0.401 a	0.374 a	0.459 a	0.317 b
Mannitol	0.813 a	0.614 c	0.703 b	0.537 d	0.620 a	0.714 a	0.758 a	0.576 b
Xylose	2.226 a	2.059 b	1.738 c	1.322 d	1.530 b	2.143 a	1.982 a	1.690 a
TSI	113.30 a	118.76 b,c	120.10 c	118.52 b,c	120.06 c	116.03 b	116.70 b	119.39 b,c

* Different letters within the same row indicate statistically significant difference at $p < 0.05$ by Tukey’s test.

Regardless of the production system, both cultivars from both production systems contained more fructose, less glucose, and the least sucrose, which is an advantage for diabetes patients, since it helps to keep the blood-sugar level constant [56]. In addition, significant amounts of galactose, trehalose, maltose, isomaltose and xylose were quantified. No matter that other sugars (arabinose, melezitose, turanose, raffinose, isomaltotriose, panose, maltotriose, gentiobiose and ribose) were present as minor constituents, some ratios should be underlined. Integrated ‘Discovery’ apples stored ~2.3-fold higher turanose and ~3.8-fold higher gentiobiose than the same fruits from organic production. Turanose (structural isomer of sucrose) is a signaling molecule, and it has been shown to accelerate fruit ripening in strawberries [57]. Gentiobiose (undesirable bitter sugar) is an osmoprotectant that stabilizes cellular membranes in water deficient conditions [58]. On the contrary, fruits from integrally produced ‘Red Aroma Orelind’ stored ~26-fold higher levels of maltotriose (an oligosaccharide which elicits a sweet taste) and ~34-fold higher levels of panose (a functional food additive due to its intestinal microflora improvement) [59,60].

The contents of all quantified sugar alcohols were below 1 mg/g and the most common sugar alcohol in all investigated apples was galactitol. Low levels or even the non-existence of sorbitol, raffinose, mannitol, and xylose were previously determined in the apple cultivars ‘Golden Delicious’, ‘Idared’ and ‘Petrovka’ from conventional production [61]. Xylose, a major neutral sugar whose accumulation is triggered by environmental stimuli, is found higher in apple fruits grown at higher altitudes [62]. In this

study, levels of this sugar were slightly higher in organic 'Discovery' and integrated 'Red Aroma Orelind' compared its counterpart, which means that it is genotype dependent. Our results correspond with the findings of Le Bourvellec et al. [63], who found sorbitol to be a relatively minor component in apple peel. There are different opinions why organic or integrated fruits should have a higher or lower sugar level. In organic production, yields are often lower, so the synthesized major and minor sugars are divided to the lower number of 'sink' organs, showing higher levels of total sugars. Contrary to that, organic fruits which are exposed to stresses could have metabolic problems and lower photosynthetic activity, and thus a lower accumulation of sugars [6,64].

3.3. Phenolic Profiles

During this study, we examined the content of certain phenolic compounds in the peel and pulp of four apple samples. The compounds of interest were mainly phenolic acids and flavonoids (aglycones and glycosides), as well as phlorizin and phloretin, which are apple-specific chalcones. A study of the phenolic profile of apple peel and pulp showed that the peel is richer compared to the pulp, in the range of ~1.2-fold (for chlorogenic acid) up to ~114-fold (quercetin 3-O-galactoside) (Table 3).

Table 3. Polyphenol profiles of peel and pulp (mg/kg) of two apple cultivars organically and integrally grown.

Phenolic Compounds	‘Discovery’ (Org.)	‘Discovery’ (Integ.)	‘Red Aroma Orelind’ (Org.)	‘Red Aroma Orelind’ (Integ.)	‘Discovery’ (Org.)	‘Discovery’ (Integ.)	‘Red Aroma Orelind’ (Org.)	‘Red Aroma Orelind’ (Integ.)
	Peel				Pulp			
Protocatechuic acid	0.53 ^{c*}	--	0.43 ^b	0.37 ^a	--	--	--	--
Aesculin	11.09 ^d	10.03 ^c	9.42 ^b	10.44 ^c	1.74 ^a	1.77 ^a	1.78 ^a	1.83 ^a
Chlorogenic acid	62.09 ^{d,e}	64.27 ^e	24.30 ^b	25.33 ^b	42.49 ^c	60.56 ^d	21.39 ^a	21.51 ^a
<i>p</i> -Hydroxybenzoic acid	1.05 ^c	0.91 ^c	0.76 ^b	1.34 ^d	--	0.32 ^a	--	--
Catechin	--	--	--	--	11.36	--	--	--
Caffeic acid	8.47 ^b	8.46 ^b	8.61 ^b	8.17 ^b	3.30 ^a	3.30 ^a	3.24 ^a	--
Syringic acid	1.49 ^a	1.84 ^b	--	1.66 ^{ab}	--	--	--	--
Rutin	--	--	11.60 ^a	46.90 ^b	--	--	--	--
<i>p</i> -Coumaric acid	0.77 ^a	1.33 ^b	1.94 ^c	0.51 ^a	--	--	--	--
Quercetin 3- <i>O</i> -galactoside	88.64 ^c	96.85 ^d	76.81 ^b	146.21 ^e	0.87 ^a	0.98 ^a	0.79 ^a	0.92 ^a
Ferulic acid	0.33 ^a	0.26 ^a	0.60 ^b	--	--	--	--	--
Naringin	--	--	0.08 ^a	0.14 ^b	--	--	--	--
Kaempferol 3- <i>O</i> -glucoside	11.72 ^e	14.33 ^f	6.19 ^c	8.30 ^d	0.27 ^a	0.42 ^b	0.23 ^a	0.23 ^a
Apigenin 7- <i>O</i> -glucoside	0.41 ^b	0.45 ^{b,c}	--	0.54 ^c	--	0.12 ^a	--	0.12 ^a
Phlorizin	67.27 ^f	46.53 ^e	33.27 ^d	29.18 ^c	1.18 ^a	2.39 ^b	2.00 ^b	0.93 ^a
Phloretin	6.91 ^c	5.94 ^b	6.68 ^c	5.62 ^b	2.00 ^a	2.01 ^a	2.01 ^a	--
Baicalein	1.21 ^c	1.08 ^b	1.26 ^c	1.00 ^b	0.23 ^a	0.24 ^a	0.24 ^a	0.23 ^a
Naringenin	2.17 ^c	1.81 ^a	2.25 ^d	1.65 ^b	--	--	--	--
Kaempferol	17.68 ^{b,c}	18.70 ^c	14.40 ^a	16.08 ^b	--	--	--	--

-- “not detected”. * Different letters within the same row indicate statistically significant difference at $p < 0.05$ by Tukey’s test.

A total of ten phenolic compounds (aesculin, chlorogenic acid, *p*-hydroxybenzoic acid, caffeic acid, quercetin 3-*O*-galactoside, kaempferol 3-*O*-glucoside, apigenin 7-*O*-glucoside, phlorizin, phloretin, and baicalein) were found both in the pulp and in the peel, with the concentrations of all ten being significantly higher in the peel.

The only compound detected in the pulp but not in the peel was catechin, quantified only in the 'Discovery' organic pulp sample at a significantly high concentration of 11.36 mg/kg. Contrary to this, Valavanidis et al. [33] found catechin in both the peel and pulp of 'Red Delicious Starking', 'Golden Delicious', 'Royal Gala', 'Granny Smith' and 'Jonagold' from organic and conventional production. A high level of catechin, which was previously quantified in the apple cultivar 'Annurca', is used to fight against the colorectal cancer cell line [65].

The compound that can be noted as dominant for the pulp was chlorogenic acid, with a concentration that ranged from 21.51 ('Red Aroma Orelind' from organic production) to 60.56 mg/kg ('Discovery' from integrated production). This acid was also found in significant amounts in the peel, in the range of 24.30 ('Red Aroma Orelind' from organic production) to 64.27 mg/kg ('Discovery' from integrated production). Our results are in agreement with those reported by Oszmiański et al. [15] and Veberic et al. [66], who proved that chlorogenic acid is the major component of apple cultivars grown in Poland and Slovenia, respectively. Generally, this phenolic acid is a precursor of flavor in fruits, and has a beneficial effect on human health, showing anticarcinogenic, antimutagenic and antioxidant [67] effects.

However, quercetin 3-*O*-galactoside was the most abundant in the peel samples [from 76.81 ('Red Aroma Orelind' from organic production) to 146.21 mg/kg ('Red Aroma Orelind' from integrated production)]. Previous studies of apple peel demonstrated that the accumulation of anthocyanin cyanidin-3-*O*-galactoside increased at low temperatures, and promoted fruit coloration by regulating anthocyanin's biosynthesis [68]. Generally, both apple cultivars had higher levels of this compound in fruits from integrated production, which is not in line with the study of Vanzo et al. [31] where Golden Delicious had a two-fold higher level of this galactoside in organic fruits compared to the integrated, but there were no differences in the cultivars 'Liberty', 'Santana' and 'Topaz'. Kaempferol (with concentration ranged from 14.40 to 18.70 mg/kg) and kaempferol 3-*O*-glucoside (with concentration ranged from 6.19 to 14.33 mg/kg) were also characteristic of the peel, both of which were higher in integrated apple fruits. Contradictory to this, Średnicka-Tober et al. [29] found no differences in kaempferol 3-*O*-glucoside between the production system in cultivars 'Champion', 'Gala' and 'Idared'. For the chalcones quantified in apple peel, we must single out phloridzin, whose concentration ranged from 29.18 ('Red Aroma Orelind' from integrated production) to 67.27 mg/kg ('Discovery' from organic production). According to Le Bourvellec et al. [63] the cultivars 'Smoothee', 'Ariane' and 'Melrose' stored dihydrochalcones as a minor group and accounted for 3% and 3.5% of the total phenolics in the pulp and in the peel, respectively, of the examined cultivars. In this study, fruits from both apple cultivars grown in organic conditions had a higher level of phloridzin, which corresponds to the study of Vanzo et al. [31] in 'Golden Delicious'. The same was true with phloretin, procatechuic acid, ferulic acid, baicalein and naringenin, whose levels were higher in peel from fruits obtained from organic production compared to the integrated production. Flavonoids (baicalein and naringenin), are produced as a defense mechanism against pathogens and abiotic stresses [69]. In humans, they are used in the prevention and treatment of vascular and cardiac disease, and cancer, while phloridzin from apple fruits is associated with potential benefits on intestinal inflammation [65,70]. Ferulic acid exhibits a vast array of functions, including its antioxidant, antiinflammatory, antimicrobial and antiallergic properties, and helps to increase the viability of sperms [71].

If we compare the tested apple cultivars, differences in the presence and absence of certain compounds were noticed. Thus, for example, rutin was found only in 'Red Aroma Orelind' samples (in organic and conventional samples) in significant concentrations

(11.60 mg/kg in organic sample and 46.90 mg/kg in conventional sample). The same is the case with naringin, which has been found in low amounts in 'Red Aroma Oreind' peel samples. Regarding pulp, no clear line could be drawn between the cultivars and the production system. Only integrated fruits from the cultivar 'Discovery' stood out with higher levels of chlorogenic acid, *p*-hydroxybenzoic acid, kaempferol 3-*O*-glucoside and phlorizin, while organic fruits from 'Reda Aroma Oreind' had higher levels of caffeic acid, phloridzin and phloretin.

This all corresponds to the previous studies where huge discrepancies regarding polyphenolic content in dessert and cider apple cultivars were found [72,73]. For polyphenols, depending on the compounds, the management has a significant effect, but it is still much lower than the effects of the cultivar [63].

3.4. Principal Component Analysis (PCA)

The multivariate analysis is a statistical technique that is used to determine the differences between the properties, which variables contribute the most to the difference and which variables are correlated with each other or completely independent from each other [74]. PCA has been already used in other studies for the comprehensive evaluation of apple fruits, juice, pomace and seed quality [23,75–78].

In this study, PCA was used to establish similarity/dissimilarity among the chemical compositions of apple samples based on the cultivars and growing conditions. Three PCA were performed separately on the polyphenols, TPC, TAC, and RSA obtained in the apple peel extracts (Figure 1A,B), polyphenols, TPC, and RSA in the apple pulp extracts (Figure 1C,D), and sugar and sugar alcohol contents in apple (Figure 2A,B). The initial matrices of four (the number of apple samples) $\times$ 21 (quantified polyphenols, TPC, TAC, and RSA in apple peel extracts), four (the number of apple samples) $\times$ 13 (quantified polyphenols, TPC and RSA in apple pulp extracts), and four (the number of apple samples) $\times$ 20 (quantified sugars) were processed using the covariance matrix with autoscaling. The PCA performed on the polyphenols contents and the results of the spectrophotometric tests obtained for apple peel and pulp extracts resulted in two-component models, which explained 82.87%, and 88.23% of total variance, respectively. The PCA score plot (Figure 1A) showed the clustering of apple peel extracts into two groups along the PC2 axis based on the significant differences in contents of polyphenols, TPC, TAC, and RSA values. From the loadings plot of PCA (Figure 1B), it was evident that kaempferol, kaempferol 3-*O*-glucoside, chlorogenic acid, and phlorizin were the most influential variables responsible for the separation 'Discovery' cultivar from 'Red Aroma Oreind'. On the other hand, those polyphenols are showing very tight correlation among each other. It is the same situation with rutin, which is closely connected with RSA and TPC, which means that total phenols and antioxidant capacity in the apple peel is rutin dependent. As for the polyphenol composition, TPC and RSA in the apple pulp extracts, the PC scores plot (Figure 2C) shows no clustering of the apple based on the cultivars and growing conditions. In the pulp, TPC depends on baicalein and kaempferol 3-*O*-glucoside, while antioxidant capacity is related to quercetin 3-*O*-glucoside.

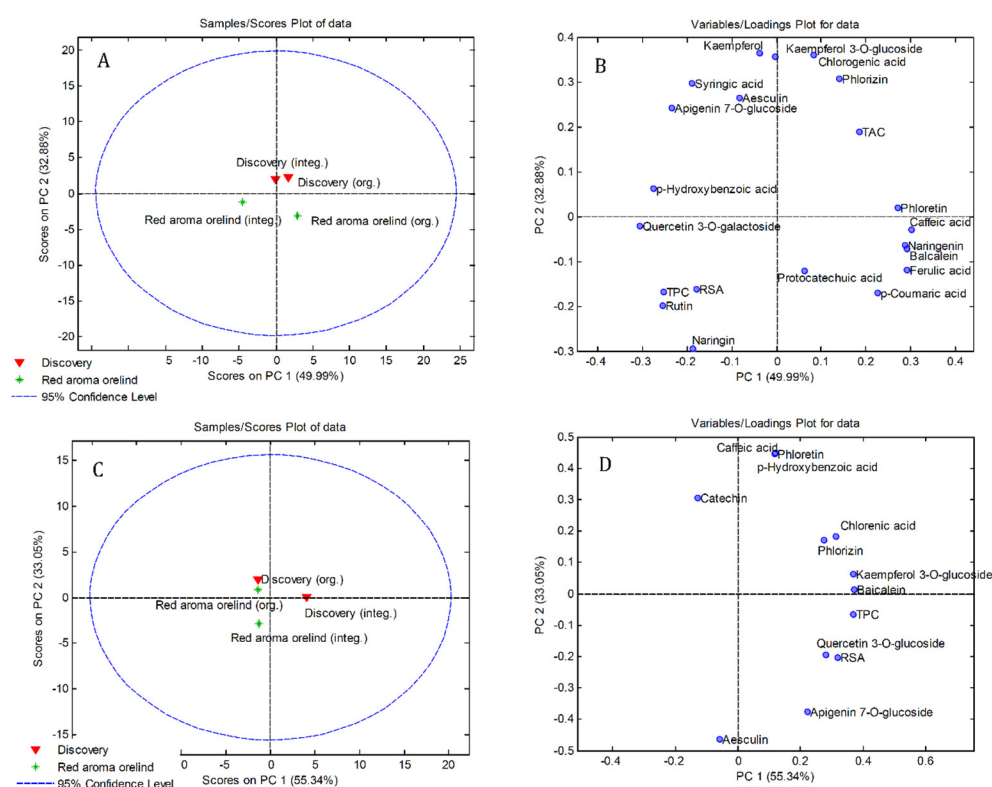


Figure 1. PCA performed on TPC, TAC, RSA and polyphenols in apple samples. PC scores plot (A) and loadings plot (B) of apple peel samples; PC scores (C) and loadings plot (D) of apple pulp extracts.

The results of PCA applied on sugar contents in apple samples suggested that the first two principal components explained 88.38% of total variance. The PCA correlation plots in Figure 2A showed that the separation ‘Discovery’ cultivar samples from ‘Red Aroma Oreilind’ along the PC1 axis. Higher contents of fructose, gentiobiose, isomaltotriose and maltotriose were the most important factors responsible for the separation of the ‘Red Aroma Oreilind’ cultivar from the ‘Discovery’ samples. On the other hand, glucose, sucrose, xylose, and raffinose had the highest negative impact on PC1, and it was the most influential in distinguishing the ‘Discovery’ cultivar.

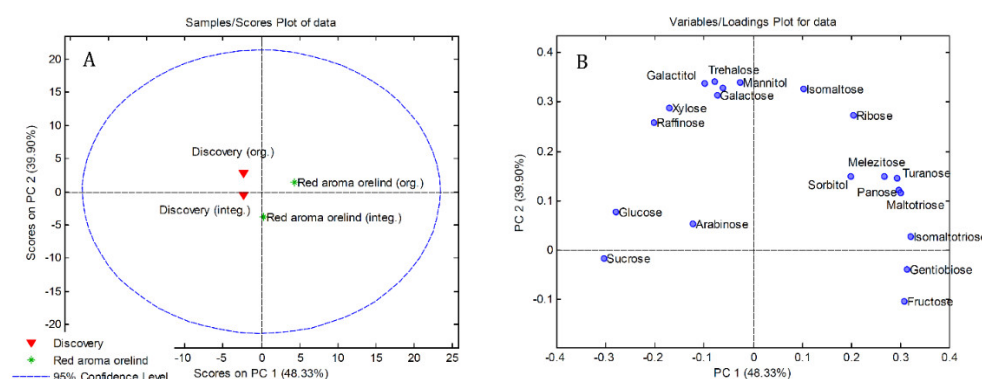


Figure 2. PCA performed on sugar and sugar alcohol contents in apple samples. PC scores plot (A) and loadings plot (B)

4. Conclusions

To the best of our knowledge, this is the first study that covers the comprehensive analysis and comparison of sugar and polyphenolic profiles from two apple cultivars

grown in organic and integrated production systems under Norwegian climatic conditions. Although we believed that organic production, as a more stressful production due to the limited mineral nitrogen and crop protection, would result in fruits with higher levels of primary and secondary metabolites, this was not the case. In relation to integrated production, apple cultivars from the organic system had higher peel TAC, glucose, sucrose, phlorizin, phloretin, protocatechuic acid, catechin, caffeic acid, *p*-coumaric acid, ferulic acid, baicalein and naringenin on average, which means that those compounds are related to the spraying program and fertilizing, whereas other practices were similar. Differences in pulp composition between the management systems were very limited, since they affected only a few minor phenolics (catechin, caffeic acid and phloretin).

Fruits from organic ‘Discovery’ drew attention due to the high RSA, TAC and phloridzin in the peel, sorbitol, glucose and sucrose, high TSI, and high levels of catechin in pulp. On the other side, fruits from integrated ‘Red Aroma Orelind’ showed high TPC and RSA of the peel, high rutin and quercetin 3-*O*-galactoside in the peel, high levels of fructose, high TSI, and several tens of times higher content of panose and maltotriose. This means that levels of bioactive compounds were different between production systems, but above all it was cultivar and fruit part dependent.

Both cultivars and both production systems in West Norway gave high quality apples, but a slight advantage should be given to organic ‘Discovery’ and integrated ‘Red Aroma Orelind’ due to the health promoting compounds and we recommend their growing in such environmental conditions

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