



Article

Assessment of Carrot Production System Using Biologically Active Compounds and Metabolomic Fingerprints

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Abstract: Carrot is the most important root vegetable plant produced worldwide, consumed as a food source rich in bioactive compounds. The purpose was to investigate the effect of different farming conditions on the content of those compounds in the roots of Afalon and Cortina carrot cv. and to use metabolomic fingerprinting for the authentication of the carrots. Carotenoids and ascorbic acid (AA) were detected and quantified using the LC method. The characterization and classification of carrot samples according to the production system, variety and plant density were performed using UHPLC-Q-TOF-MS. The metabolomic fingerprints/profiles were measured in positive and negative ionization mode on a reversed-phase analytical column, and the data were processed using the relevant statistical tools. The respective AA levels in Afalon and Cortina were 217–291 and 318–514 mg.kg^{−1} dry biomass. The main carotenes were β - and α -carotene whose ratio in Afalon and Cortina was 1.3 and 1.2, respectively. A set of 19 compounds detected by UHPLC-Q-TOF-MS, selected on the basis of their importance for statistical distribution into individual groups representing a farming system, cultivar, or seeding density, was established and used for the authentication of the carrot samples. These compounds included various saccharides, phenolics, and lipids with antioxidant, anti-inflammatory, anticancer, and antibiotic activities. The metabolomic data obtained were used for the verification of the farming system in which the carrots were grown.

Keywords: production system; carrot cultivars; seeding density; ascorbic acid; carotenoids; metabolomics fingerprinting; statistical analysis; *Daucus carota* L.



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1. Introduction

Carrot (*Daucus carota* L.) is a root vegetable widely produced in the world that serves as an important source of dietary fiber and common nutrients, such as sugars and minerals, and of various bioactive compounds, including ascorbic acid (AA), carotenes, phenolics, and polyacetylenes, for the human diet [1]. In the Czech Republic, the carrot belongs to the main vegetable products, representing more than 7% of the total vegetable sowing surface [2]. A number of review papers on carrot have covered the effect of various preharvest factors, such as genetic background, soil and climatic conditions, farming conditions, nitrogen level, fertilization, pest attacks, etc., that influence the quality characteristic of the vegetable [3,4].

The demand for organically grown vegetables has recently increased on the market and this trend will probably continue as organic products are believed to be of better quality and taste, containing high amounts of health-beneficial compounds, smaller levels of nitrite, and no residues of pesticides [3,5–7]. On the contrary, conventional production aims at maximal crop yields and economic efficiency, using industrial fertilizers and pesticides [8]. Regarding carrots, it was found that the amount of nitrite was greatly

decreased in organic production, while the content of AA was only slightly decreased, and the differences between the levels of β -carotene, polyacetylenes, and phenolics in organically and conventionally grown carrots were not significant [9–12]. In this work, the integrated farming system was also designed to produce high-quality vegetables using available ecological methods that sought to minimize the supply of agrochemicals [13].

AA, carotenoids, and phenolics belong to compounds with health-promoting effects due to their antioxidant activity [14]. Different carrot cultivars show broad ranges of AA, carotenoid, and phenolic contents varying between about 20–800 mg.kg^{−1} DW biomass, 150–400 mg.kg^{−1} FW biomass, and 160–1020 mg.kg^{−1} FW biomass, respectively [15–20]. The contents of β - and α -carotenes were reported to be highly cultivar-dependent [21,22]. Generally, high rates of nitrogen fertilization were shown to have adverse effects on the content of AA but increased the levels of carotenoids in carrot [3,23–25]. The amount of phenolics in carrots was found to vary broadly depending on the cultivar but was quite stable in different farming systems [22,26]. Carrot contains mainly hydroxycinnamic acids and their derivatives, of which chlorogenic acid as a major component represents 42–62% of the total phenolic compounds [14].

Other biologically active compounds present in carrot that improve human health comprise polyacetylenes exhibiting antitumor, anti-inflammatory, antibacterial, and antifungal effects. Falcarinol, falcarindiol, and falcarindiol-3-acetate are the main polyacetylenes found in carrot roots [4,27–29]. The amount of polyacetylenes was found to be cultivar-dependent. Their content was affected by the chemical composition of soil, namely by low concentrations of P, Mg, and Ca, but the differences between the organic and conventional carrots were not significant [26,30–32]. The carrot was reported to also be a source of ferulic acid that exhibits antimicrobial and health-beneficial properties [33,34]. 6-methoxymellein (6-MM), a biologically active phytoalexin, is accumulated in carrot roots and leaves in response to a fungal infection and is also considered to have anticancer properties [35–37]. Its presence has been associated with abiotic stress [38,39].

The quality control and the authenticity of food has become an urgent matter. An objective assessment of a product quality based on good scientific knowledge is needed. The advantage of using metabolomics for food authentication consists of its untargeted nature that enables us to detect changes in a typical metabolic profile resulting from inappropriate production practices [40]. Metabolite fingerprinting approaches have been repeatedly used to investigate differences in crops produced in organic and conventional farming systems, including various cereals, vegetables, or fruits [41–45]. The results were inconclusive as in some studies only small differences were detected [44], whereas in others, significant differences were found, e.g., [41,42]. Major influences of the crop year and cultivar on the metabolic profile were reported [44,46]. In the case of carrot, the production year was the most important source of variation in the crop metabolome [47]. The results indicated that the farming system had a systematic influence on the metabolome of carrots. Using the Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) based on LC-MS detection, the carrot samples could be classified according to the farming system and the origin of unknown samples could be predicted. The method was able to classify unknown samples harvested within each crop year, achieving 100% correctness [47].

The aim of our study was to investigate the influence of farming conditions on the levels of bioactive compounds in carrots. Two carrot varieties, Afalon and Cortina, were grown under organic, integrated, and conventional conditions in one locality, using two different planting densities of 6×10^5 and 9×10^5 seeds per hectare. Biologically active compounds such as carotenoids and ascorbic acid were quantified using LC-based techniques and expressed as the dry matter content. The study focused on assessing the utilization of metabolomic fingerprinting/profiling for the authentication of carrots. The characterization and classification of the carrot samples according to their production system, variety, and plant density were carried out using Ultra-High-Performance Liquid Chromatography coupled to a Quadrupole–Time-of-flight Mass Spectrometer (UHPLC-Q-TOF-MS). The metabolomic fingerprints/profiles were measured in positive and negative ionization mode

on a reversed-phase analytical column. To calculate the statistical models, the obtained data were processed using the relevant statistical tools (PCA, PLS-DA, and OPLS-DA models).

2. Materials and Methods

2.1. Biological Material

In the study, two carrot (*Daucus carota* subsp. *sativus*) cultivars (cvs.), Afalon and Cortina, were used. Afalon F1 is a semi-late hybrid cv. of the transition type, suitable for mechanized harvest, whose vegetation time is 115–120 days. Cortina F1 is a late hybrid cv. of the Flakkee type, suitable for mechanized harvest, whose vegetation time is 150–160 days. Both cvs. are widely used for commercial production of carrot in the Czech Republic and wider eastern Europe.

2.2. Farming Conditions

The experiments were conducted in the experimental station of the Czech University of Life Sciences in Prague-Troja (Czech Republic). The locality has an altitude of 195 m above sea level and is characterized by dry, medium-warm climatic conditions with a soil classified as modal fluvisol (pH 6.9).

The conventional system (conv) used the following fertilization conditions: 180 kg of nitrogen per ha (urea before seeding 80%, ammonium nitrate with limestone after sprouting 20% (LAV, Agro CS, Říkov, Czech Republic)). Chemical protection was (per ha): STOMP—pendimethalin (BASF AG, Agricultural Products, Ludwigshafen/Rhein, Germany), 2 L; GARLAND FORTE—propaquizafop (Dow AgroSciences, Prague, Czech Republic), 1 L; ORTIVA—azoxystrobin (Syngenta Limited, Cambridge, UK), 1 L; FUNGURAN—OH 50 WP (Spiess Urania Chemicals GmbH, Hamburg, Germany); PIRIMOR 50 WG—pirimicarb (Syngenta Supply AG, Basel, Switzerland), 1 kg.

The integrated system (int) used the following fertilization conditions: 120 kg nitrogen per ha (urea before seeding 80%, ammonium nitrate with limestone after sprouting 20% (LAV, Agro CS, Říkov, Czech Republic)). Chemical protection was (per ha): STOMP (BASF AG, Agricultural Products, Ludwigshafen/Rhein, Germany), 3.5 L; ORTIVA—azoxystrobin (Syngenta Limited, Cambridge, UK), 1 L; and PIRIMOR 50 WG—pirimicarb, 0.5 kg.

The organic system (org) used no chemical protection, and the fertilization conditions included the use of 42 kg nitrogen per ha in the form of Organica (LAV, Agro CS, Říkov, Czech Republic) organic fertilizer.

In the experimental plots, the sowing densities were 6×10^5 (LD) and 9×10^5 (HD) seeds ha^{-1} . The latter density is commonly used for commercial production, whereas the former was used for comparison to achieve better light conditions and a reduced level of natural humidity in the plant cover. Each cultivar in every crop system was grown in four replicates. The size of the experimental plot for each replicate was 9 m^2 . From each replicate, three carrot roots were collected, which made (per variant) 3 roots \times 4 replicates = 12 roots. This was applied for each cultivar and each crop system.

2.3. Chemical Analyses

2.3.1. AA Analysis

The AA content in biomass was measured using a modified method of [48]. An amount of 30 g of fresh carrot biomass was extracted with 60 mL of metaphosphoric acid (Fluka, Germany) (3%; *w/w*) by homogenization in a laboratory blender (Ultra-Turrax IKA-T10 basic, IKA, Staufen, Germany) at laboratory temperature. The homogenate was filtered, adjusted to a volume of 100 mL, and filtered again (5 μm membrane filter, Chromservis, Prague, Czech Republic) into vials. AA was detected using HPLC with UV detector (liquid chromatograph HP 1200 with DAD detector, Agilent Technologies, Santa Clara, CA, USA). LiChroCART, LiChrospher 100 RP-18 (Merck, Darmstadt, Germany) chromatographic column (125 \times 4 mm; particle size 5 μm) with guard column (4 \times 4 mm; particle size 5 μm) was used with a mobile phase containing 5% methanol (gradient grade, Sigma-Aldrich, Taufkirchen, Germany) (V/V), pH = 3 (ortho-phosphoric acid, Lachner, Neratovice,

Czech Republic), and deionized water (18 M Ω cm, Milli-Q system, Millipore, Darmstadt, Germany). The other conditions were: flow rate 0.8 mL min⁻¹, temperature 35 °C, injection volume 5 μ L. The UV detection was carried out at 244 nm.

The identification of AA in the samples was carried out by comparing the retention time with that of the standard (L-ascorbic acid, Sigma Aldrich, Steinheim, Germany, purity \geq 99%). For quantification, an external calibration curve was used. The characteristics of the method were the following: repeatability expressed as RSD 5%, recovery 95%, and LOQ 0.15 mg kg⁻¹ dry weight (DW).

Dry carrot biomass was determined by drying at 105 °C for 5 h and used to express the AA and carotene contents per g dry mass.

2.3.2. Carotene Analysis

The α - and β -carotene content was analyzed in fresh carrot samples using a modified method of Bhavé et al. [49]. Carotenes were extracted by an ethanol/hexane mixture (1:1; v/v) containing 0.2% BHT and annealed sodium sulfate (Lachner, Neratovice, Czech Republic) (3 g per 1 g of biomass) under shaking (30 min) followed by centrifugation (10,000 rpm, 5 min). Ethanol and *n*-hexane were purchased from Merck, Darmstadt, Germany, and BHT was purchased from Sigma-Aldrich, Taufkirchen, Germany. The upper hexane layer was removed and the ethanolic portion was re-extracted with hexane three times. The combined hexane extracts were evaporated at 30 °C (Büchi Labortechnik AC, Nettetal, Germany) and the dry residue dissolved in an acetone/ethanol (4:6; v/v) mixture containing 0.2% BHT and subsequently filtered (0.22 μ m centrifugation PTFE microfilters, Chromservis, Prague, Czech Republic) into a vial. The samples were protected against light during extraction, stored in dark vials, and analyzed immediately after extraction.

An Agilent Technologies 1200 HPLC with DAD detector (liquid chromatograph HP 1200, Agilent Technologies, Santa Clara CA, USA) device equipped with a visible-light photodiode-array detector was used to analyze the samples. The chromatographic separation was carried out using an analytical column Poroshell 120 EC-C18 100 \times 2.1 mm; particle size 2.7 μ m at a column temperature of 30 °C. The mobile phases were (A) water and (B) methanol (MeOH) with gradient elution and a flow rate of 0.5 mL.min⁻¹. The DAD measurements were recorded in the UV and visible regions (190 to 800 nm), with the quantification at 450 nm for α - and β -carotene and 444 nm for lutein.

The β -carotene (purity \geq 99%, Sigma-Aldrich, Taufkirchen, Germany) and lutein (purity \geq 95%, Labicom, Czech Republic) standards were prepared by dissolving an amount of 4–5 mg in 25 mL of an acetone/ethanol (4:6, v/v) mixture containing 0.2% BHT and the solution was used for preparation of a calibration curve in the range of 0.2–20 μ g.mL⁻¹. The LOQ was 1.5 mg.kg⁻¹. The method was validated, and the repeatability expressed as the relative standard deviation (RSD was 5% for α - and β -carotene, 3% for lutein, calculated from 6 parallel measurements). Due to the instability of carotenoids, a fresh standard was prepared prior to each measurement and protected against light. The α -carotene content was quantified using the β -carotene standard.

2.3.3. Metabolomic Fingerprinting

An amount of 5 g of the homogenized sample was extracted with 5 mL of a solvent (methanol for polar compounds and ethylacetate for less polar compounds extraction (both were from Sigma-Aldrich, Prague, Czech Republic)) under vigorous shaking for 2 min and subsequently centrifuged (10,000 per min, 5 min, 5 °C; Rotina 38R, Hettich Zentrifugen, Tuttlingen, Germany). The upper layer was filtered through a 0.22 μ m PTFE microfilter prior to LC-MS analysis.

The UHPLC–Q-TOF-MS analyses were performed using an Acquity Ultra-Performance LC system coupled to a Synapt G2 HD spectrometer (Waters, Milford, MA, USA). The LC separation was performed by an Acquity UPLC[®] BEH C18 column (100 \times 2.1 mm; particle size 1.7 μ m; Waters, Milford, MA, USA). The mobile phase consisted of A) 10 mM ammonium formate in Milli-Q water and B) 10 mM ammonium formate in methanol. The

gradient started at 95% A and linearly changed to 95% B in 8 min and was held for the next 4 min, and the initial conditions were set for the last 3 min to allow column reconditioning. The flow rate was 0.4 mL.min⁻¹ and 3 µL were injected into the system.

The Synapt G2 HD instrument was operated in both negative and positive electrospray ionization (ESI) mode. The parameters settings used during the measurements were as follows: capillary voltage (±700 V), cone voltage (±25 V), source temperature (120 °C), and desolvation temperature (350 °C). Nitrogen was used as both desolvation and cone gas at a flow rate of 800 and 10 L.h⁻¹, respectively. The data were acquired in MS^E acquisition mode, both the full MS and MS/MS fragmentation mass spectra were acquired in the range *m/z* 100–1200. In order to diminish any possible time-dependent changes in the UHPLC-MS chromatographic fingerprints, the sequence of the samples was randomized, and one sample was chosen as a quality control sample, which was injected after every set of 24 samples. A processing blank was prepared together with samples and analyzed for the exclusion of the co-extracted compounds during sample preparation (e.g., plasticizers from plastic cuvettes, etc.). MassLynx 4.1 software (Waters, USA) was used for data acquisition and its application manager MarkerLynx was used for data mining and pre-processing.

2.3.4. Chemometric Analysis

From the UHPLC-MS data, markers were chosen automatically by the MarkerLynx software. The raw data in the form of absolute peak intensities (heights) were normalized using the constant row sum, i.e., each variable was divided by the sum of all variables for each sample. This procedure transformed all the data to a uniform range of variability.

The SIMCA v. 16.0.1. software (Sartorius, Goettingen, Germany) was then used for multivariate data analysis. Pareto scaling (square root of the standard deviation is used as the scaling factor) was applied prior to the analysis.

The Principal Component Analysis (PCA) was employed as an unsupervised chemometric technique for the first view into data structure; Partial Least Squares Discriminant Analysis (PLS-DA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) were used as a supervised technique for the classification of samples according to their classes (variety, farming system, and seeding density). Variables important for separation were selected and a tentative identification was suggested. The model performance was expressed by the R²(X) parameter, known as ‘goodness of fit’, and by the Q², the predictive ability parameter. Both parameters were calculated using a 7-fold internal cross-validation by SIMCA software.

3. Results and Discussion

3.1. Content of AA and Carotenoids

The organic, integrated, and conventional farming systems were used at one locality to avoid different climate and soil conditions. The systems differed in the amounts of nitrogen used and in the application of pesticides (see Section 2.2). The respective values of dry biomass of Cortina and Afalon cv. grown in the three farming systems fluctuated between 11.1 and 13.0% (*w/w*), with a higher average content in Cortina (12.5%) in comparison with Afalon (11.8%). The data are comparable with our previous study [50] and also with the study by [51] where the dry matter content of fresh carrots in two consecutive years was around 12% (*w/w*). Bioactive compounds in carrots were detected and their amounts measured using LC-based techniques with DAD detection and expressed as dry matter content.

The AA content was highly cultivar-dependent; higher AA levels were found in Cortina compared to Afalon cv., regardless of the farming system and the seed density applied (Figure 1a,b). On average, a higher content of AA was determined in the former cultivar by up to 40%, and the differences between the cultivars were statistically significant ($\alpha = 0.05$). The AA levels were similar to the values reported by Alasalvar et al. [22] (444 mg/kg DW) and Gamboa-Santos et al. [52] (356 mg/kg DW). Nitrogen fertilization used at a high rate has repeatedly been reported to decrease the AA amounts in vegetables,

such as carrot, tomatoes, broccoli, and cauliflower [23,25,53]. In our case, where N concentrations ranged between 42 and 180 kg.ha⁻¹, no adverse effect of a high N concentration on the AA content was observed in either cultivar. Generally, a slightly higher AA content was recorded in the samples grown at a lower planting density (Figure 1a,b). A low variability of AA levels between various farming systems was observed in Afalon. The highest AA levels of about 514 mg.kg⁻¹ DW were measured in the Cortina samples in the organically produced carrot grown at 6×10^5 seed density and in the integrated system-produced carrot grown at 9×10^5 seed density (Figure 1a,b).

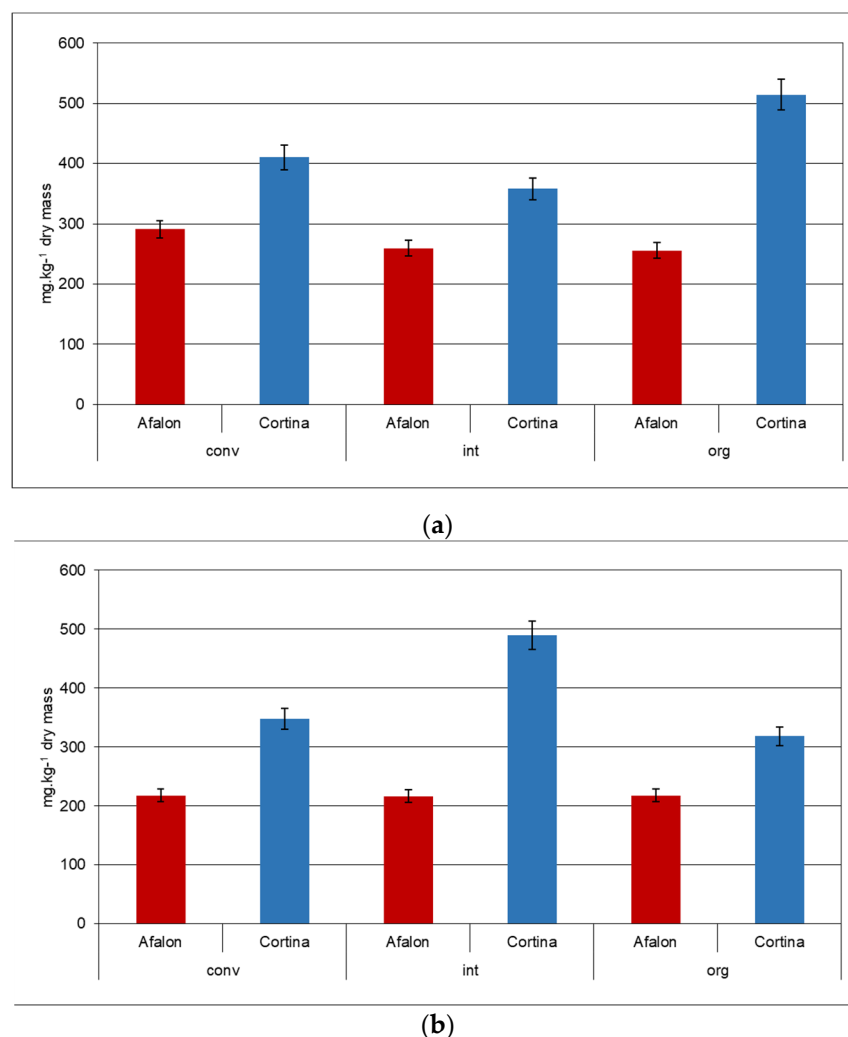


Figure 1. Content of AA in Cortina and Afalon cv. produced in conventional (conv), integrated (int), and organic (org) systems: (a) seeding density 6×10^5 seeds.ha⁻¹; (b) seeding density 9×10^5 seeds.ha⁻¹. The amount of AA is expressed in mg per kg DW.

The main carotenoids measured in the carrot samples of both cultivars were β -carotene (469–714 mg.kg⁻¹ DW) and α -carotene (382–733 mg.kg⁻¹ DW); only low levels of lutein (4.8–8.0 mg.kg⁻¹ DW) were detected. The ratios of β - to α -carotene in Afalon and Cortina cv. were in the range of 1.3 and 1.2, respectively (Figure 2a,b). Some previous studies showed that increased rates of nitrogen fertilization in the range of 0 to 640 kg N.ha⁻¹ had a positive effect on the carotene levels in carrot [54,55]. On the contrary, Smoleň and Sady [17], working with the Kazan F1 carrot cv. and using nitrate or ammonia N-sources, documented that an increase in the range of 70–140 and 70–210 kg N.ha⁻¹, respectively, resulted in a negligible effect on the total carotene levels monitored in the crop year period of 2003–2005. Boskovic-Rakocevic et al. [25] reported an increase of 1.2- and 1.3-fold in

Nantes carrot cv. in crop years 2005 and 2006, when the N-fertilization rate was increased from 0 to 180 kg N.ha⁻¹. They concluded that an increase in carotene content was linked with the increasing N-fertilization.

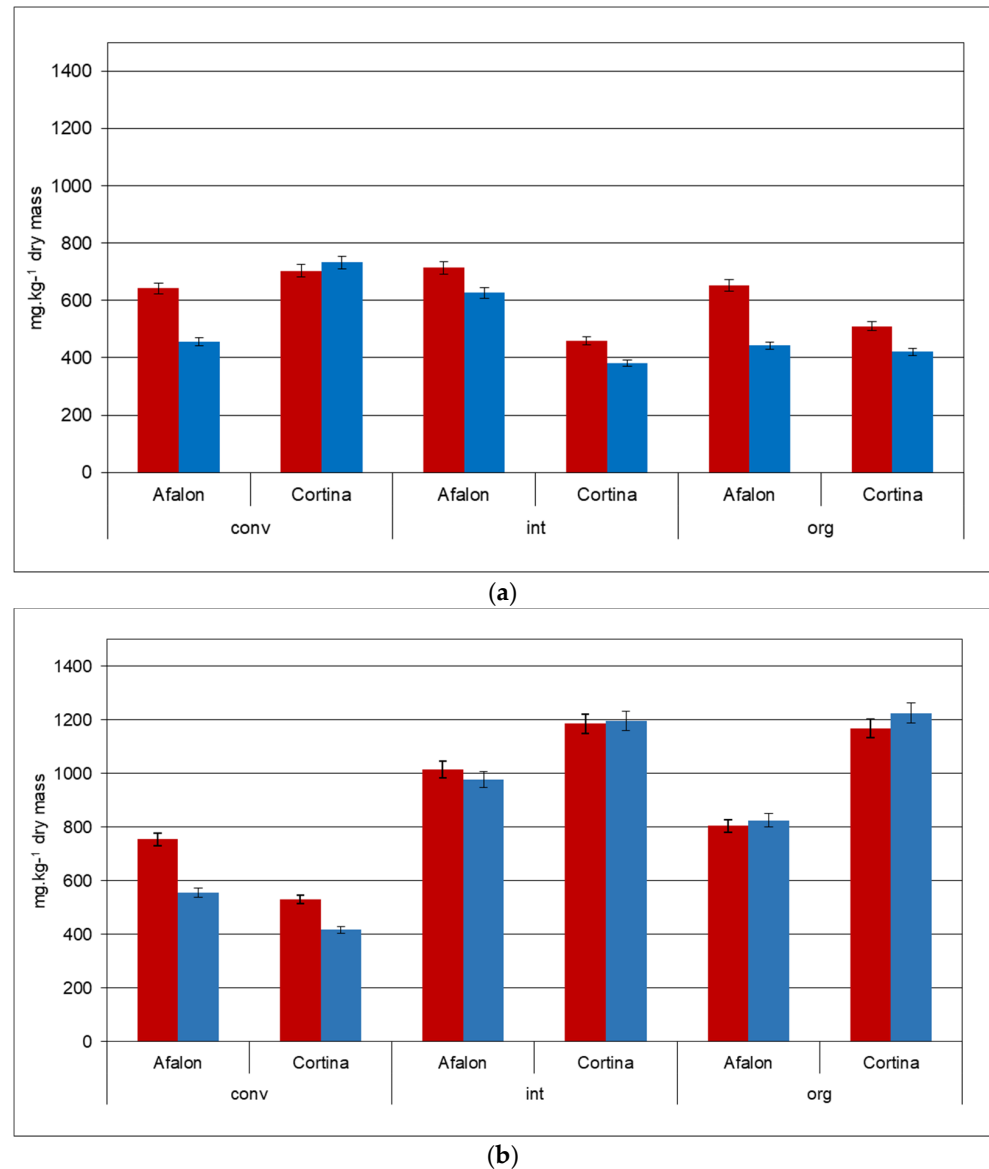


Figure 2. Content of β -carotene and α -carotene in Cortina and Afalon cv. Produced in conventional (conv), integrated (int), and organic (org) systems: (a) seeding density 6×10^5 seeds.ha⁻¹; (b) seeding density 9×10^5 seeds.ha⁻¹. The level of carotenes is expressed in mg per kg DW; β -carotene, red; α -carotene, blue.

In our study, such an effect was observed in Afalon cv., where the carotene content was raised 1.3-fold when comparing the carrot grown in the organic (42 kg N.ha⁻¹) and integrated (120 kg N.ha⁻¹) farming systems at both seeding densities. In Cortina cv., this trend was observed only at a seeding density of 6×10^5 where the carotene content increased 1.6-fold, when comparing the carotene contents obtained in the organic (42 kg N.ha⁻¹) and conventional (180 kg N.ha⁻¹) farming systems (Figure 2a). In the integrated farming system using 120 kg N.ha⁻¹, the carotene level of Cortina cv. remained at a low level observed in the organically grown carrot (Figure 2a).

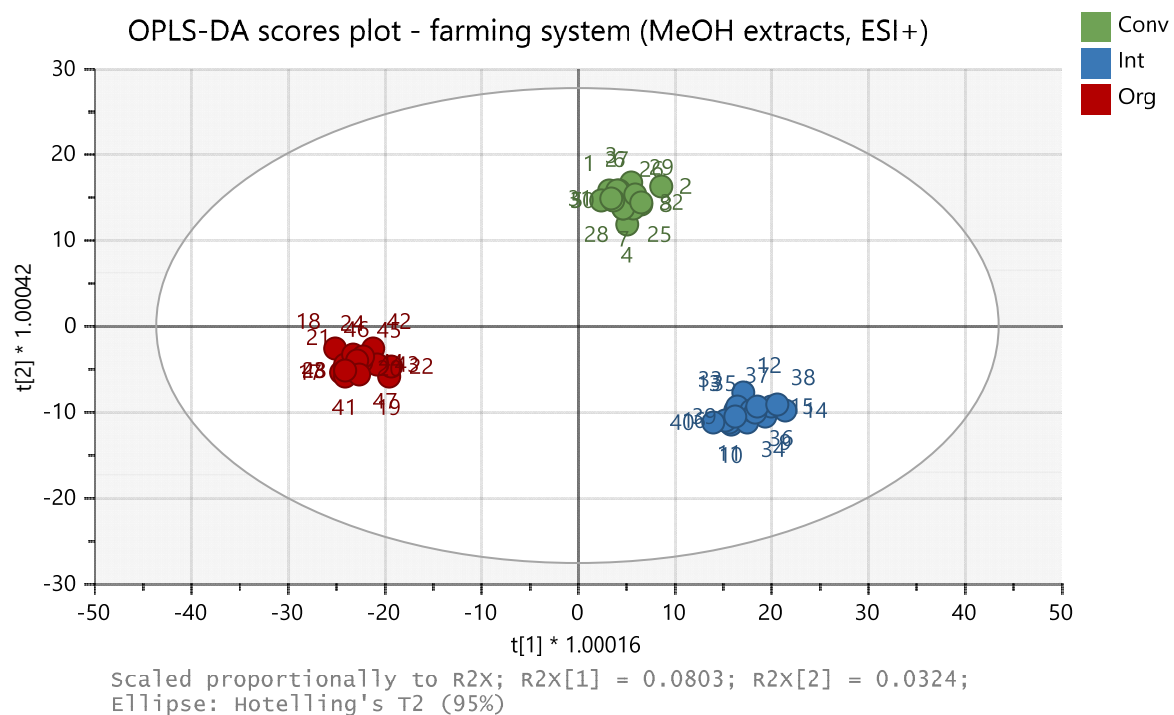
When the seeding density of 9×10^5 seeds.ha⁻¹ was used, the production of total carotene in the conventional system was reduced compared to the other two farming

systems in both cultivars (Figure 2b). Thus, the results demonstrated the effect of both the cultivar and seeding density on the carotene synthesis and accumulation.

3.2. Metabolomic Fingerprinting

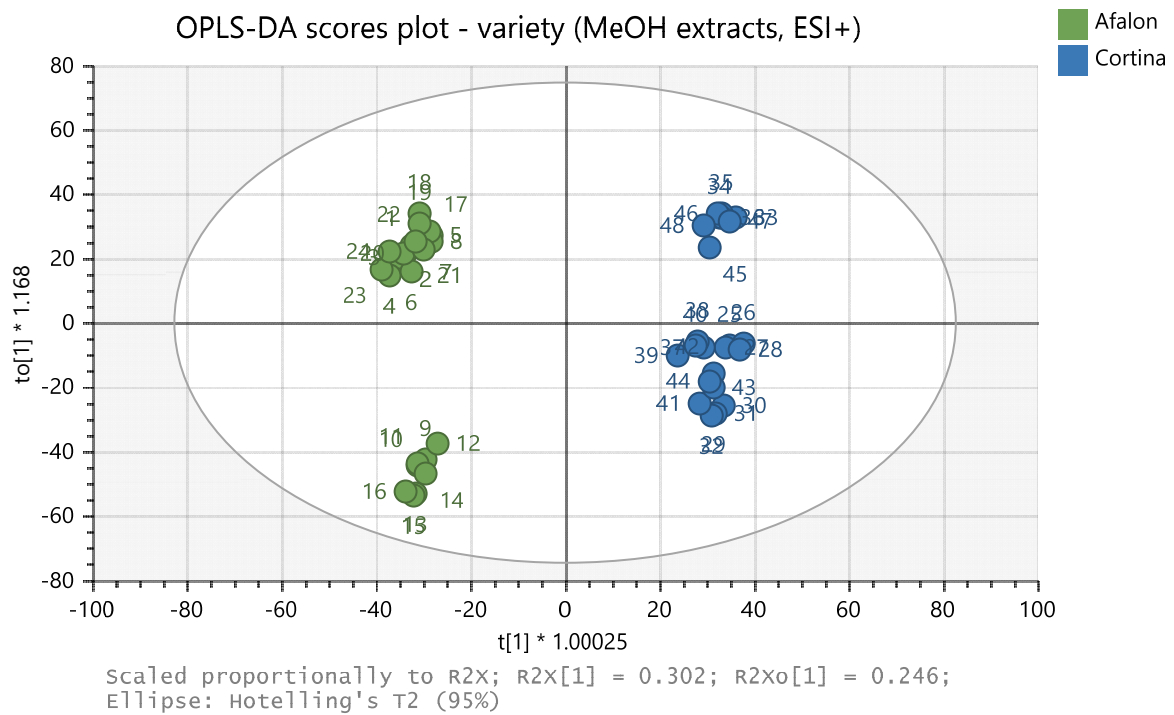
The carrot samples were characterized and classified according to their production system, variety, and plant density by untargeted metabolomics using UHPLC–Q-TOF-MS with data-independent acquisition. This method of data acquisition, the so-called MS^E, provides exact-mass precursor and fragment mass spectra for every detectable component in the sample extract, and this is very useful for compound identification. Furthermore, a retention time obtained by the reversed-phase chromatographic separation gave additional information about the hydrophobicity of the detected compounds.

For the methanolic extract, 216 and 38 features (ions defined by their exact mass and retention time) were chosen as variables for the data processing in positive and negative mode, respectively. For the ethylacetate extracts, 1274 and 125 features were chosen in the positive and negative mode, respectively. The carrot samples were successfully differentiated according to the farming system, cultivar, and the seeding density used by the application of a non-target analysis using the statistical models PLS-DA and OPLS-DA. Examples of the OPLS-DA plots are presented in Figure 3a–c for the farming system, variety, and planting density, respectively. The model performance parameters are listed in Table 1; a better classification of the samples was observed for the methanolic extracts in the positive ionization mode. The metabolomic profiles were mostly influenced by variety.

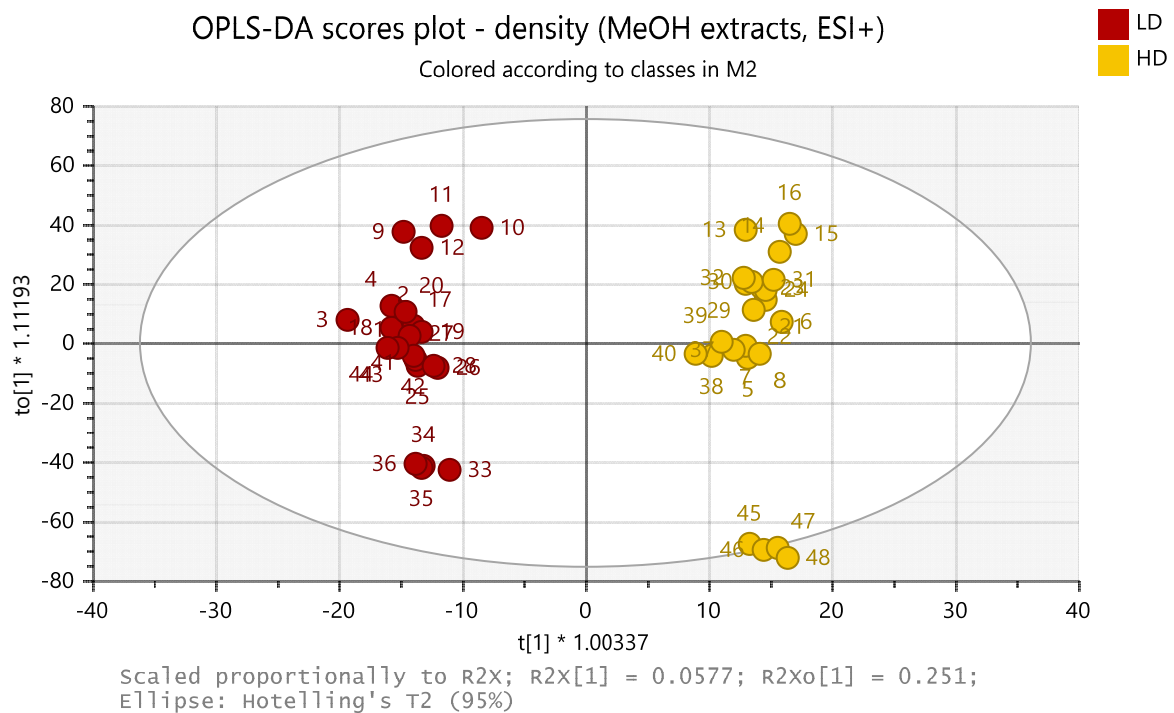


(a)

Figure 3. Cont.



(b)



SIMCA 16.0.1 - 07.02.2022 11:42:06 (UTC+1)

(c)

Figure 3. OPLS-DA model of 48 samples (methanolic extracts, positive ion mode). The samples are clustered according to: (a) farming system (conv = conventional, int = integrated, org = organic), (b) variety, and (c) planting density (LD = low density, 6×10^5 seeds per ha, HD = high density, 9×10^5 seeds per ha).

Table 1. An overview of the performance parameters of the OPLS-DA models.

Extract (Ionization)	Farming System (Organic vs. Integrated vs. Conventional)	Variety (Cortina vs. Afolon)	Seed Density 6×10^5 vs. 9×10^5 Seeds.ha ⁻¹
MeOH (+)	$R^2(X) = 0.97$ $Q^2 = 0.92$	$R^2(X) = 0.99$ $Q^2 = 0.99$	$R^2(X) = 0.98$ $Q^2 = 0.88$
MeOH (−)	$R^2(X) = 0.82$ $Q^2 = 0.54$	$R^2(X) = 0.99$ $Q^2 = 0.98$	$R^2(X) = 0.97$ $Q^2 = 0.92$
EtOAc (+)	$R^2(X) = 0.98$ $Q^2 = 0.85$	$R^2(X) = 0.99$ $Q^2 = 0.96$	$R^2(X) = 0.98$ $Q^2 = 0.92$
EtOAc (−)	$R^2(X) = 0.97$ $Q^2 = 0.87$	$R^2(X) = 0.99$ $Q^2 = 0.96$	$R^2(X) = 0.97$ $Q^2 = 0.89$

Identification of Important Ions

The identification of the characteristic ions was suggested according to their exact mass, isotopic profile, and fragmentation spectra obtained by the acquisition in MS^E mode, which were compared with those included in the databases Metlin, PubChem, and mzCloud. The compounds were selected on the basis of their importance for the (O)PLS-DA distribution into the individual groups, representing the farming system, the cultivar, or the seeding density (Variable Importance for the Projection; VIP), and were used for the authentication of carrot samples. The identified compounds are listed in Tables 2 and 3 for methanolic and ethylacetate extracts, respectively. Most of them were important for the sample discrimination; the importance of the markers for the individual above-mentioned groups is summarized in the last column, referred to as ‘Importance for Group’, i.e., the marker showing a higher intensity in the respective group. The identification was revealed based on the literature [29,56,57] and on the results obtained in our previous study [50]. The compounds included various saccharides, polyphenolic compounds, and lipids. Many of them were compounds with important biological activities, e.g., antioxidants (chlorogenic acid and ferulic acid), anti-inflammatory and anticancer activity (chlorogenic acid, ferulic acid, falcariol/falcarindiol, and apiole), antibiotic activity (ferulic acid, octadecanoic acid, falcariol/falcarindiol and apiole), and pesticide activity (falcariol/falcarindiol), or were found to contribute to the plant resistance to fungal pathogens (linolenic acid) [37,58–62].

The effect of the farming system on the synthesis and accumulation of biologically active compounds included in the metabolomic fingerprinting was best demonstrated on the levels of 6-MM and apiole, metabolites with antimicrobial properties [1,36,37], whose amounts were characteristically increased in Afolon cv. in the integrated production system, compared to the organic and conventional system levels. In Cortina cv., the amounts accumulated in the carrot roots also differed between the individual production systems but were strongly affected by the seeding density.

The most important compounds that differentiated the two cultivars studied were falcariol (RT = 8.70; m/z = 245.1901 [M + H]⁺; higher in Cortina), trihydroxyoctadecenoic acid (RT = 5.77; m/z = 353.2300 [M + Na]⁺/329.2322 [M − H][−]; higher in Afolon), and the compound with the formula C₁₈H₂₄O₁₂ (RT = 2.17; m/z = 433.1343 [M + H]⁺/431.1201 [M − H][−]; higher in Cortina). This compound was identified as Licoagroside B, although it was not previously identified in carrot. However, the fragment spectra match those in the PubChem database and in the literature [63,64]. Chevalier et al. [65] also reported this compound as a discriminating factor for carrot varieties; however, the authors reported only the molecular formula without further identification. In Cortina, the effect of the seeding density was rather low, but it was much more pronounced in the Afolon, where higher levels of Licoagroside B were detected at the higher seeding density.

Table 2. List of compounds identified by UHPLC–Q-TOF-MS used for metabolomic analysis of carrot samples (methanol extracts). The compounds are sorted according to their retention time. (Conv = conventional, int = integrated, org = organic, LD = low seeding density, 6×10^5 seeds per ha, HD = high seeding density, 9×10^5 seeds per ha).

MeOH extracts in positive ionization mode						
RT [min]	Formula	Identification	Δ ppm	$[M + H]^+$	Fragments Obtained	Importance for Group
0.61	C ₁₂ H ₂₂ O ₁₁	Sucrose	−1.17	365.1054 [M + Na] ⁺	203.0509	No clear trend
1.64	C ₁₂ H ₁₆ O ₈	Glucosylmaltol	0.64	311.0740 [M + Na] ⁺	127.0391	HD
2.17	C ₁₈ H ₂₄ O ₁₂	Licoagroside B	−0.70 −1.20	433.1343 455.1160 [M + Na] ⁺	127.0390	Cortina, Conv
4.49	C ₁₂ H ₁₄ O ₄	Apiole	2.24	223.0965	205.0860	Int, Conv
5.33	C ₁₁ H ₁₂ O ₄	6-methoxymellein	1.96	209.0814	191.0704; 181.0860; 163.0759; 135.0806; 103.0554	Afalon, Int
5.77	C ₁₈ H ₃₄ O ₅	Trihydroxy-octadecenoic acid	−1.11	353.2300 [M + Na] ⁺	213.1474	Afalon
7.63	C ₂₁ H ₂₆ O ₇	Laserine/epilaserine	−1.75	413.1569 [M + Na] ⁺	291.1228	Int, HD
7.75	C ₂₁ H ₂₆ O ₇	Laserine/epilaserine	−1.75	413.1569 [M + Na] ⁺	291.1228	Cortina
7.96	C ₂₅ H ₃₈ O ₅	Non-identified	−1.80	441.2609 [M + Na] ⁺	341.1750; 283.1671; 161.1324	Conv, Afalon
MeOH extracts in negative ionization mode						
RT [min]	Formula	Identification	Δ ppm	$[M - H]^-$	Fragments obtained	Importance for group
0.54	C ₆ H ₈ O ₇	Citric acid	−0.93	191.0185	111.0074	Afalon
0.60	C ₅ H ₁₀ N ₂ O ₃	Glutamine	−1.50	145.0605		LD
0.63	C ₁₂ H ₂₂ O ₁₁	Sucrose	−1.17	341.1081	179.0544	No clear trend
1.43	C ₁₆ H ₁₈ O ₉	Chlorogenic acid	−1.86	353.0866	191.0551	Afalon
2.17	C ₁₈ H ₂₄ O ₁₂	Licoagroside B	2.67	431.1201	369.1672; 329.0829; 269.0706; 125.0233	Cortina, Conv
3.93	C ₁₄ H ₂₆ O ₅	hydroxytetradecanedioic acid	0.37	273.1702	255.1592; 155.0700	Afalon
4.22	C ₁₀ H ₁₀ O ₄	Ferulic acid	0.08	193.0494	133.0269	Cortina
5.41	C ₁₈ H ₃₂ O ₅	Trihydroxyoctadecenedioic acid	−2.29	327.2164		HD
5.75	C ₁₂ H ₂₂ O ₄	Dodecanedioic acid	−6.48	229.1425	211.1332	Afalon
5.77	C ₁₈ H ₃₄ O ₅	Trihydroxy-octadecenoic acid	−1.82	329.2322		Afalon, HD
8.68	C ₁₈ H ₃₂ O ₂	Linoleic acid	2.13	279.2330		Cortina

Table 3. List of compounds identified by UHPLC–Q-TOF-MS used for metabolomic analysis of carrot samples (ethylacetate extracts). The compounds are sorted according to their retention time. (Conv = conventional, int = integrated, org = organic, LD = low seeding density, 6×10^5 seeds per ha, HD = high seeding density, 9×10^5 seeds per ha).

EtAOc extracts in positive ionization mode						
RT [min]	Formula	Identification	Δ ppm	$[M + H]^+$	Fragments Obtained	Importance for group
4.41	C ₁₂ H ₁₄ O ₄	Apiole	2.24	223.0965	190.0622; 177.0911; 163.0791; 147.0437; 133.0670	Cortina
5.72	C ₁₁ H ₁₀ O ₄	Eugenin	−2.58	207.0652	195.1379; 107.0852	Afalon, Int, LD
7.34	C ₁₅ H ₂₀ O ₂	Eremofrullanolide	−1.72	233.1540	217.1579; 159.1635; 119.0848	Cortina
7.62	C ₁₈ H ₃₀ O ₂	Linolenic acid	−2.15	279.2320	191.0694; 161.1319	Int
7.63	C ₂₁ H ₂₆ O ₇	Laserine/epilaserine	−1.75	413.1569 $[M + Na]^+$	291.1239; 259.1665; 159.1170	Int
8.12	C ₁₇ H ₂₄ O ₂	Falcarindiol	−0.76	261.1852		No clear trend
8.70	C ₁₇ H ₂₄ O	Falcarinol	−1.63	245.1901		Cortina
EtAOc extracts in negative ionization mode						
RT [min]	Formula	Identification	Δ ppm	$[M - H]^-$	Fragments obtained	Importance for group
5.74	C ₁₈ H ₃₄ O ₅	Trihydroxyoctadecenonoic acid	0.03	329.2328	229.1432; 211.1326; 183.1368	Afalon, Org, HD
7.60	C ₁₈ H ₃₂ O ₃	Hydroxyoctadecadienoic acid	−0.23	295.2277	277.2177; 195.1385	Int, Afalon
8.25	C ₁₆ H ₃₂ O ₃	Hydroxypalmitic acid	−3.68	271.2267	225.2209; 223.2040	Cortina, Conv, HD
8.69	C ₁₈ H ₃₂ O ₂	Linoleic acid	−5.01	279.2316		Cortina

The effect of seeding density was clearly demonstrated for the falcarinol levels in both cultivars. The levels of eremofrullanolide and ferulic acid were also strongly affected by the seeding density in both cultivars and in all the farming systems used. The amount of the former compound was cultivar-dependent, being higher in Cortina cv. in all the farming systems. Interestingly, in terms of the level of eremofrullanolide in both cultivars, the effect of the seeding density was different in the conventional system compared to the integrated and organic systems. In the conventional system, the higher density of 9×10^5 resulted in higher amounts of eremofrullanolide in contrast to the other two farming systems, where the trend was the opposite. The effect of the seeding density on the levels of ferulic acid was similar in both cultivars in the integrated and organic farming systems; the lower seeding density increased the level of ferulic acid in the integrated system but suppressed it in the organic system.

4. Conclusions

The effect of various farming conditions on the quality of carrots using two carrot varieties was investigated in organic, integrated, and conventional farming systems. The

AA content was highly cultivar-dependent, regardless of the farming system and the seeding density. An increase in carotene content with increasing N-fertilization was observed; however, the synthesis and accumulation of carotenoids were strongly affected by both the cultivar and seeding density used. The metabolomic fingerprinting/profiling method was used to assess the authenticity of the carrots. The UHPLC-QTOFMS method followed by a multivariate data analysis enabled us to differentiate between organic, integrated, and conventionally produced carrots, as well as the carrot varieties or the seeding density applied. A set of compounds, including saccharides, phenolics, and lipids with antioxidant, anti-inflammatory, anticancer, and antibiotic activities, selected according to their importance for statistical distribution into individual groups representing farming system, cultivar, or seeding density was established and used for carrot authentication. The metabolome was mainly influenced by the cultivar used; however, the effect of the farming conditions was also documented. Metabolomic data were used for the verification of the farming system where the carrots were grown.

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