Volatile compounds and selected qualitative parametres of apples from organic and integrated farming – can we evaluate the difference?

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Summary

The more the shops are flooded by apples from organic and integrated farming, the more the desire of markets and consumers to confirm the way of their treatment grows. The question how to distinguish between organic and integrated production inspired this research. Differences between apples from organic and integrated farming were investigated by optimized headspace solid-phase microextraction-gas chromatography-mass spectrometry analysis. Together 34 samples representing 10 apple cultivars from 6 locations in the Czech Republic, 2 production systems and 2 harvest years were analysed. Approximately 70 volatile compounds were detected, 45 of them were chosen for statistical processing, 33 were identified. The highest contents were observed for 1-butanol, 2-methyl 1-butanol and 1-hexanol. Higher soluble solids values of 10 apple varieties noticed in 2015 harvest compared to 2016 indicated possible connection to 2015 dry seasonal conditions. In contrast, higher acidity was obviously related to cultivars Topaz, Melodie and Ontario. Anyhow, the distinction between organic and integrated production was not noticed for 2015 varieties but, for 2016, a shift towards production system differentiation was observed. Differences between seasonal conditions of harvest years 2015 and 2016 probably represented the main limiting factor for distinction between apples from organic and integrated farming.

Besides conventionally produced food commonly offered in stores, organic and integrated production represent popular targets of consumers' interest. While conventional production respects certain low levels of contaminants according to legislative limits, organic and integrated production follow more restrictive criteria. The plant organic production is connected to stricter legislative rules defined in particular in Regulations (EC) No. 848/2018 [1], No. 889/2008 [2] and No. 1235/2008 [3]. Together with high demands on food safety and quality, it cares to maintain the highest level of organic matter in soil as well as soil's fertility and biological activity or to prevent its compaction and erosion [4, 5]. Integrated production represents an intermediate step between organic and conventional agriculture. It does not allow the use of plant protection products with non-specific effects, prescribes control of soil and fruit samples for heavy metals as well as monitoring of climate indicators and pests every 7 months [4]. According to stricter criteria for application of protective preparations and respecting of prescribed agrochemical procedures, higher hygienic-toxicological and nutrient quality of apples coming from organic or integrated farming is expected. Anyhow, the characteristics of apples can evoke questions about their qualitative aspects as well as whether their production under strictly defined rules is controlled. The content of specific volatile secondary metabolites and toxic substances or other technological and sensory

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quality feature may be important indicators for such evaluations [4].

Formation of secondary metabolites is a subject of interest of metabolomics, analytical procedure focusing on the detection of a wide spectrum of small molecules in complex biological matrices and the interpretation of reactions leading to their formation [6]. For instance, direct immersion-solid-phase microextraction two-dimensional gas chromatography-time-of-flight mass spectrometry (DI-SPME-GC×GC-TOF-MS) technique was well adapted for applications of metabolomics, when scientists identified 399 metabolites in apples according to RISTICEVIC et al. [7]. According to the study of ROWAN [8], plants produce a number of metabolites in increased amounts to ensure specific biological functions as attracting pollinators, providing defence against pests and pathogens or serving as important flavouring compounds detectable by humans. Moreover, studies of RAFFO et al. [9, 10] indicated that growing conditions with reduced use of nitrogen fertilizers, which are typical conditions for organic production according to 889/2008 [2], might be connected to formation of higher levels of some volatile secondary metabolites in comparison with conventional fertilization treatments richer in nitrogen. The studies mention that the levels of butyl acetate and hexyl acetate, which significantly contribute to the aroma of apples, or hexyl butyrate [10], tended to be higher in organic apples from trees that were not fertilized. Conversely, apples from the trees treated with nitrogenous fertilizers, producing less butyl acetate and hexyl acetate, had higher levels of alcoholic precursors butanol, hexanol and 2-methyl butanol [9]. Simultaneously, according to both studies [9, 10], apples produced without fertilization were characterized by lower levels of C6-aldehydes 1-hexanal and (*E*)-2-hexenal compared to the fertilized counterparts. However, based on literature, the differences in volatiles formation need not be directly caused by diverse production types. LE BOURVELLEC et al. [11] compared the influence of management systems (organic, low-input and conventional), yearly conditions and cultivar on metabolite profile and qualitative parametres of apples. The authors found out that the management system appeared to have a lower impact on the profile of fruit metabolites than the cultivar and year had. The study of ROTH et al. [12], focusing on comparison between different storage conditions, stated that the quality attributes of apples coming from different production systems (organic, integrated) did not differ significantly, neither at harvest nor after storage. Beneficial finding was made in the

study of NERI et al. [13] concluding that thawed apples after freezing and frozen storage showed the same browning independently on the agricultural farming method (organic, conventional). Pretreatments, freezing and frozen storage similarly affected the apples of both agricultural systems, but organically grown apples showed higher mechanical strength after processing than their conventionally grown counterparts.

In the above studies, production of secondary metabolites was described as a result of various factors. Enzymatic activity plays a key role in formation of such volatile metabolites. Alcohol-acyl-transferase (AAT), the enzyme generating volatile esters by esterification of alcohols, or lipooxygenase (LOX) enabling production of esters, aldehydes, alcohols and fatty acids through fatty acids oxidation, belong to those most discussed [14, 15]. Alcohol dehydrogenase (ADH) and pyruvate decarboxylase are often mentioned according to their responsibility for conversion of acetaldehyde to ethanol and pyruvate to acetaldehyde [16]. Thus, it can be hypothesized that factors such as year, with its changeable seasonal climatic conditions, the farming system influencing the growing conditions, or diverse apple cultivars and their different adaptation to local conditions [17], may have significant effects on enzyme activity and formation of volatile compounds. The influence of these factors on overall qualitative attributes including sweetness and acidity is also in question. Evaluation of impact of such factors might contribute to assessment of possibility to authenticate apple cultivation conditions. Due to the lack of experimental data in this field in literature, their extension was more than desirable. Therefore, to evaluate the factors' impact, it was focused on statistically evaluable differences among volatile compounds and among qualitative parametres of individual apple varieties in relation to discussed factors.

MATERIALS AND METHODS

Materials

Together 34 different apple samples were provided by the Czech University of Life Sciences Prague (Prague, Czech Republic). Samples coming from organic and integrated production from 6 locations in the Czech Republic distant from each other not more than 130 km were harvested in 2 years, 2015 and 2016 (Tab. 1). Apples were harvested in stable climatic conditions during each year's harvest period, gradually according to the harvest ripeness, which was determined after many

Tab. 1. Tested apple varieties.

Sample	Apple variety	Harvesting year	Place	Geographic coordinates
Integrated production				
AI5	Angold	2015	Praha-Suchdol	50°7'59'' N, 14°22'36'' E
AI6	Angold	2016	Holovousy, district Jicin	50°22'31'' N, 15°34'39'' E
FI5	Florina	2015	Praha-Suchdol	50°7'59'' N, 14°22'36'' E
FI6	Florina	2016	Praha-Suchdol	50°7'59'' N, 14°22'36'' E
GI5	Gloster	2015	Praha-Suchdol	50°7'59'' N, 14°22'36'' E
Gol5	Goldstar	2015	Praha-Suchdol	50°7'59'' N, 14°22'36'' E
Gol6	Goldstar	2016	Praha-Suchdol	50°7'59'' N, 14°22'36'' E
II5	Idared	2015	Praha-Suchdol	50°7'59'' N, 14°22'36'' E
II6	Idared	2016	Praha-Suchdol	50°7'59'' N, 14°22'36'' E
MI5	Melodie	2015	Praha-Suchdol	50°7'59'' N, 14°22'36'' E
OI5	Ontario	2015	Praha-Suchdol	50°7'59'' N, 14°22'36'' E
OI6	Ontario	2016	Praha-Suchdol	50°7'59'' N, 14°22'36'' E
RI5	Rubín	2015	Praha-Suchdol	50°7'59'' N, 14°22'36'' E
RI6	Rubín	2016	Melnik Chloumek	50°22'22'' N, 14°30'23'' E
SI5	Champion	2015	Praha-Suchdol	50°7'59'' N, 14°22'36'' E
TI5	Topaz	2015	Praha-Suchdol	50°7'59'' N, 14°22'36'' E
TI6	Topaz	2016	Praha-Suchdol	50°7'59'' N, 14°22'36'' E
Organic (bio) production				
AB5	Angold	2015	Borohradek, Sachov	50°5'50'' N, 16°7'13'' E
AB6	Angold	2016	Borohradek, Sachov	50°5'50'' N, 16°7'13'' E
FB5	Florina	2015	Borohradek, Sachov	50°5'50'' N, 16°7'13'' E
FB6	Florina	2016	Borohradek, Sachov	50°5'50'' N, 16°7'13'' E
GB5	Gloster	2015	Radim, district Chrudim	49°54'16'' N, 16°1'19'' E
GoB5	Goldstar	2015	Borohradek, Sachov	50°5'50'' N, 16°7'13'' E
GoB6	Goldstar	2016	Borohradek, Sachov	50°5'50'' N, 16°7'13'' E
IB5	Idared	2015	Radim, district Chrudim	49°54'16'' N, 16°1'19'' E
IB6	Idared	2016	Radim, district Chrudim	49°54'16'' N, 16°1'19'' E
MB5	Melodie	2015	Borohradek, Sachov	50°5'50'' N, 16°7'13'' E
OB5	Ontario	2015	Borohradek, Sachov	50°5'50'' N, 16°7'13'' E
OB6	Ontario	2016	Radim, district Chrudim	49°54'16'' N, 16°1'19'' E
RB5	Rubín	2015	Radim, district Chrudim	49°54'16'' N, 16°1'19'' E
RB6	Rubín	2016	Borohradek, Sachov	50°5'50'' N, 16°7'13'' E
SB5	Šampion	2015	Radim, district Chrudim	49°54'16'' N, 16°1'19'' E
TB5	Topaz	2015	Borohradek, Sachov	50°5'50'' N, 16°7'13'' E
TB6	Topaz	2016	Borohradek, Sachov	50°5'50'' N, 16°7'13'' E

years of experience with the varieties studied, on the basis of good stem-to-fruit separability, with the typical skin colouration of well-developed fruits at the seed-brown stage. Apples of the same varieties from organic and integrated production were harvested at approximately the same time in both 2015 and 2016. For the Champion and Melody varieties, it was in the first decade of September, for Angold and Rubín in mid-September, for Florina in late September, for Gloster, Goldstar and Topaz in the first decade of October and for Idared in mid-October. The method of agrotechnics did not significantly affect the date of harvest. In both years 2015 and 2016, no industrial fertilizers, organic fertilizers or synthetic pesticides for plant protection were used in the organic planting of apple trees on vigorous rootstocks and within the entire ecological orchard. The supply of soil with humus was good due to the entire area of grassing the orchard. None of the insecticides allowed in organic production (for example, NeemAzal produced by Biocont Laboratory, Brno, Czech Republic, which was used in the previous period against aphids), were used during that period. Also, the infectious pressure of fungal diseases in relatively dry periods was very weak, so it was not necessary to apply fungicides. In the case of integrated production, the apple samples were grown in the system maintained by cutting grass of intermediate rows with additional irrigation. The chemical protection was carried out in a gentle manner according to the rules of

the Association for Integrated Fruit Production SISPO (Holovousy, Czech Republic), no organic fertilizers were used. In both years 2015 and 2016, nitrogenous fertilizers were applied from mineral nutrients at a rate of N 60 kg·ha^{-1}, phosphorus at a rate of P 70 kg·ha^{-1} and potassium at a rate of K 70 kg·ha^{-1}. Fungicide treatment against major fungal diseases (scab and mildew) during the growing season (2015 and 2016) in accordance with methodological guidelines for plant protection was performed on average 5 times with active substances mancozeb (preparations Dithane 3 kg·ha^{-1} and Novozir 3 kg·ha^{-1}), dodine (Syllite 1.5 kg·ha^{-1}), copper (Kuprikol 4 kg·ha^{-1}) and sulfur (Cumulus 5 kg·ha^{-1}). Twice during the vegetation period in both years, insecticide interventions against pests (aphids, codling moths) with the active substances pirimicarb (Pirimor 0.4 kg·ha^{-1}) and thiacloprid (Calypso 0.25 kg·ha^{-1}) were necessary. In addition, glyphosate (Roundup herbicide 4 kg·ha^{-1} calculated area) was applied to the grown weeds once every year in the belts under the treetops.

All 34 apple samples were cut into eighths immediately after harvesting, cored and stored in closed plastic packages in a freezer (−18 °C) until the beginning of analytical procedures (12–24 months). Apple juice Pfanner 100% (made from concentrate; Hermann Pfanner Getränke, Lauterach, Austria) serving as the quality control (QC) sample and Golden Delicious trial apple samples for optimization procedure were purchased in a supermarket in the Czech Republic. Chemicals NaCl (p.a.), NaOH (p.a.) and formaldehyde solution (p.a., 36–38 %) were obtained from Penta (Prague, Czech Republic). Analytical standard for titrimetry potassium hydrogen phthalate (p.a. ≥ 99.5 %) and standards 1-butanol (≥ 99.5 %), (+/−)-2-methyl-1-butanol (≥ 98 %), hexanal (98 %), ethyl butyrate (≥ 98 %), butyl acetate (≥ 98 %), ethyl 2-methylbutyrate (99 %), *trans*-2-hexen-1-al (≥ 95 %), 2-hexen-1-ol (96 %), 1-hexanol (≥ 99 %), 2-methylbutyl acetate (natural, ≥ 95 %), butyl butyrate (98 %), ethyl hexanoate (≥ 99 %), hexyl acetate (99 %) and hexyl butyrate (≥ 98 %) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA), a subsidiary company of Merck (Darmstadt, Germany). Helium (purity 99.998 %) was supplied by Linde Gas (Prague, Czech Republic).

Climatic conditions

According to the Report on the environment of the Czech Republic 2015 [18], the year 2015 was exceptionally above normal in terms of temperature in the Czech Republic, the average annual temperature of 9.4 °C was 1.9 °C higher than the 1961–1990 normal. Significantly above-average temperatures were recorded during the summer months. In July, up to 18 tropical days were recorded. August, extremely above normal with a deviation of 4.9 °C from normal, was the warmest recorded since 1961. The average temperature for the summer months June–August (19.2 °C) was, after the year 2003, the second highest in the observation period since 1961. In terms of rainfall, 2015 was strongly below normal. The annual total rainfall was the second lowest since 1961, the average annual rainfall of 532 mm represented 79 % of the 1961–1990 normal. The sum of surface rainfalls for the period from January to July was the lowest since 1961 (289 mm) with rainfall in February and July strongly below normal. According to the Report on the environment of the Czech Republic 2016 [19], the average 2016 annual temperature of 8.7 °C was 1.2 °C higher than the normal in 1961–1990. Temperature in February was extremely above normal (+4.1 °C), the months of June, July and September were very warm or warm. In terms of rainfall, 2016 was a normal year in the Czech Republic. However, compared to normal, the months of August and December were dry, when only about 53 % and 56 % of the monthly normal rainfall fell, while above-normal rainfall was recorded in February, July and October. The rainfall in 2016 was unevenly distributed in the Czech Republic, the deficit in the annual total was mainly in Eastern Bohemia. More details are presented in supplemental material.

Soluble solids and titratable acidity

The determination of soluble solids and titratable acidity was performed for all tested apple varieties from integrated and organic farming to check and compare the basic qualitative parameters. Several grams of each apple sample were mixed by Ultraturrax Yellow line, DI 25 basic (IKA-Werke, Staufen, Germany) to a homogenous mash. A representative amount (approximately 4 g) was placed in the refractometer DR301-95 (A. Krüss Optronic, Hamburg, Germany) and the content of soluble solids was recorded [20]. To measure titratable acidity, approximately 10 g of each mashed apple sample was mixed with approximately 40 ml of distilled water and titrated with 0.25 mol·l^{-1} NaOH by Method 8 on a titrator DL22 Food and Beverage Analyzer (Mettler-Toledo, Columbus, Ohio, United States) [21]. The values of soluble solids were recorded twice and averaged. The titratable acidity was calculated twice according to two measurements performed for each apple variety and averaged results were expressed as grams of malic acid per kilogram of the sample.

Optimization, sample preparation and isolation of volatiles

The preparation procedure and basic isolation steps (as well as chromatographic conditions described later) were modified for solid samples from the methodology used for the determination of aroma compounds in apple juices [22]. Optimizization procedure was performed on a small series of the Golden delicious apple trial samples using separation by headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS) analysis. The measured volatiles content was monitored depending on the amount of apple sample for analysis, mixing time and mixing speed. For the real sample analysis, approximately 20 g of each apple sample were cut into small pieces, put in a glass beaker and mixed to homogenous consistency with 30 g of NaCl and 60 g of distilled water for 5 min at a mixing speed of 130 Hz using Ultraturrax Yellow line DI 25 basic. Five grams of each sample were transferred into a vial (10 ml glass vial; Supelco, Bellefonte, Pensylvania, USA), and similarly 5 ml volumes of apple juice Pfanner to prepare QC samples. A standard solution with concentrations of standards of 350 μg·l^{-1}, serving for check of analytical accuracy of measurements, was prepared by diluting standards in distilled water. Four mililitres of the prepared solution were placed into a vial with 1.5 g of NaCl. All vials were sealed with 1.3 mm thick PTFE/silicone septum and 18 mm thread magnetic stainless steel screw cap. HS-SPME was performed with 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco) and autosampler Combi Pall CTC Analytics (Agilent Technologies, Santa Clara, California, USA). The fibre was pre-conditioned by blank (distilled water) injection prior to the analysis. For the extraction, samples were incubated for 1 min at 30 °C. Then, the fibre was kept for 30 min in the headspace at the same temperature. NaCl contained in samples helped to increase volatiles compounds extraction efficiency in the headspace thanks to its salting-out effect. During both procedures (incubation and extraction), the sample was agitated (4 Hz).

Analysis of volatiles

The gas chromatograph System 7890A coupled to inert mass selective detector with triple-axis detector 5975C (Agilent Technologies) was used. Chromatographic separation was performed on column DB-5 (5%-phenyl-methylpolysiloxane; 30 m × 25 μm × 0.25 μm; Agilent Technologies), using helium as a carrier gas with a flow rate of 1.2 ml·min^{-1}. Volatile compounds were thermally desorbed for 2 min in the injector port of the chromatograph at a temperature of 250 °C. The sample was injected in a split mode with a split ratio of 10 : 1. The oven was held at the initial temperature of 60 °C for 2 min, then the temperature increased to 250 °C with a ramp rate of 10 °C·min^{-1}, overall analysis took 24 min. The mass spectrometer was operating in a scan range (m/z) of 40–550 amu, with temperature of the ion source of 230 °C and temperature of quadrupole of 150 °C. To avoid large loss of volatiles during the long period in the autosampler, no more than 9 apple samples, 1 QC sample, 1 standard solution and 5 blanks were involved in one cycle of measurement. Each analysis was carried out within a series of 3 apple varieties (Tab. 1) and 3 samples of each variety. The blank was placed at the beginning of the sequence and after each 3 samples. A standard solution with concentrations of standards 350 μg·l^{-1} and QC sample of apple juice were measured at the end of each sequence to check the analytical accuracy. Identification of volatile compounds was carried out by comparison of their mass spectra with Mass Spectral Library NIST 11 (National Institute of Standards and Technology, Gaithersburg, Maryland, United States) incorporated in GC-MS data analysis software MSD ChemStation (version G1701EA E.02.02.1431, Agilent Technologies). R match of 800 was considered as a bottom threshold for each correctly identified metabolite. To validate the correct identifications, the elution order of the volatiles in our measurement conditions were compared with the order of Retention Indices (RI) of the identical volatiles on DB-5 column according to the database NIST WebBookChemie SRD 69 [23]. The exception of co-elution of ethyl butyrate with hexanal at retention time of 3.06 min represented the limiting factor in estimating the peak areas of these two volatiles. Repeatability (expressed as relative standard deviation) was finally determined for each identified substance from 3 aliquots of each measured apple sample. Alcohols 1-butanol, 2-methyl-1-butanol and 1-hexanol provided repeatabilities of up to 5 %, aldehydes hexanal and 2-hexenal of 10–12 %, esters of 15–30 %. The reason for higher repeatability values of esters could be in their lower stability and low content resulting in inaccurately read data.

Statistical methods

The computer program Statistica 12 (StatSoft, Tulsa, Oklahoma, USA) was applied for data evaluation by ANOVA Type V regarding variance estimation and precision analysis (VEPAK) and multivariate statistical methods, namely, clus-

ter analysis (CA) and discriminant analysis in its classic version, which is optional in the software. The level of uncertainity was 0.05. The analysis was performed on the basic database created from 45 evaluable apple volatiles, 33 of them were identified according to NIST 11 and on the basis of their retention indices (Tab. 2–5). Statistical evaluation was performed with peak areas of 14 selected volatiles of the database by univariate ANOVA analysis and multivariate discriminant analysis and confirmed by multivariate cluster analysis applied to the complete database of 45 volatile compounds.

RESULTS AND DISCUSSION

More than 70 volatile compounds were detected in the chromatographic spectrum set to integrate volatiles with the minimum peak area of 500 (expressed as abundance × second) according to GC-MS data analysis software used. The findings on particular volatiles raised the question of whether the increased amounts or absence of specific compounds might help to verify organic production conditions or to distinguish between different conditions of cultivation. At first glance at the results, it is worth noting that variety FB6 contained five or more times more hexyl acetate than the other varieties (Tab. 2–5). Considering the results in the studies of RAFFO et al. [9, 10], the higher level might represent proof of reduced use of nitrogen fertilizers for this organically produced variety. Anyhow, to accept such a finding as a proof of maintenance of organic conditions, more similar conclusions should be available in literature. Similarly, only two apple cultivars from organic production (Goldstar and Idared) did not contain bromodichloromethane (varieties GoB6 and IB6; Tab. 3, Tab. 5), which is typically found as originating from chlorinated drinking water [24]. It is worth considering if such a finding could contribute to assess maintenance of organic growing conditions, as non-chlorinated water was probably used for cultivating these two samples. Simultaneously, ethyl-2-methyl-butyrate was found only in organically grown cultivars Ontario, Rubín and Topaz (varieties OB5, RB6, TB6). However, its content was low in comparison with levels of dominant volatiles (Tab. 2–5). Therefore, it is questionable whether the reason of its presence could be in organic farming conditions. Further, several of the volatile compounds were found in a single sample or rarely (Tab. 2–5). However, it is hard to estimate if those are specific for certain apple va-

rieties or are specific for the farming system or are a results of other factors.

This study did not focus on enzyme activity, so its contribution to formation of volatile metabolites is difficult to evaluate based on the data obtained. Anyhow, a deeper look to this problematics can help to better clarify absence of several volatile compounds in the results (Tab. 2–5). Acetate esters were not prominent among the other volatiles in a large number of apple varieties, namely butyl acetate and hexyl acetate were absent in 94 % and 74 % of the samples, respectively. Thus, considering the studies of LARA et al. [14] and ECHEVERRÍA et al. [15] mentioning that the composition of esters (with special focus on butyl, hexyl and 2-methylbutyl acetate) could be controlled by the selectivity and activity of the enzymes AAT and LOX or by the substrate availability, low activity of these enzymes or low availability of the necessary alcohol precursors could cause low (undetectable) levels of acetate esters. Regarding the high levels of hexyl and butyl acetate precursors 1-butanol and 1-hexanol, those substrates previously considered as the cause of several-fold higher levels of acetate esters [15], lack of enzymatic activity seems to be a more probable reason for low levels of acetate esters in our study. This assumption is consistent with the finding of LARA et al. [14], where inhibition of AAT activity in combination with low LOX activity resulted in diminution of biosynthesis of volatile esters. Considering the study of DEFILLIPPI et al. [25], presenting the rise of AAT activity after treatment by ethylene, and the study of DUNEMANN et al. [26], mentioning the influence of the ethylene-dependent ripening process on the final levels of aroma compounds, possible low availability of ethylene could contribute to the reduced AAT activity thus generating lower levels of acetate esters. With regard to the study of DEFILIPPI et al. [25], deficiency of 2-hexenal in several tested varieties (undetectable, Tab. 2, Tab. 3) could follow low accumulation of its fatty acid precursor linolenic acid during ripening. Simultaneously, other enzymes influencing production of aldehydes (e.g. ADH, LOX) should be considered. However, the assumption made above considering the influence of enzymes and precursors on levels of volatile compounds should be taken as theoretical.

Closer look at the results and differences caused by diverse factors reveals that the year of production represented the factor having the strongest impact (Tab. 6, Tab. 7, Fig. 1, Fig. 2). Regarding this fact and as the folowed sentences discuss in more detail, the differences between climatic conditions of each year apparently rep-

Tab. 2. Results GC-MS analysis of apple varieties from integrated production 2015.

Volatiles	RT [min⁻¹]	Apple samples									
		Al5	Fl5	Gl5	Gol5	Il5	Ml5	Ol5	Rl5	Sl5	Tl5
		Peak area (abundance × second)									
Not identified	1.49	59134	33264	83309	29927	41015	71680	48755	44277	ND	37369
Not identified	1.58	20005	42757	24806	12906	45091	8116	20011	6078	8966	7440
Not identified	1.69	38279	11417	23005	27377	18360	4904	14421	18602	18348	16494
Not identified	1.78	27430	41089	32190	43573	39754	26037	25824	15284	41963	27377
1-Butanol	1.92	3013417	967226	562650	1607343	1118254	1585391	1567945	1011763	1482185	460469
Pentanal	2.13	24315	19505	23532	24917	9674	16183	15153	18574	16906	14815
Bromodichloromethan	2.25	11643	11419	10136	17402	7970	18449	10582	4311	12994	8823
2-Methyl-1-butanol	2.44	805008	2187126	1504943	88518	331360	17921	1045590	71142	73130	41112
1-Pentanol	2.68	76108	49831	52875	38908	10486	13630	53268	20379	28158	10951
Hexanal + ethyl butyrate*	3.06	32460	26756	25179	121452	19244	14721	20788	36801	50879	20090
Not identified	3.12	9552	16060	5083	12596	13710	8950	9786	7603	9527	8087
Butyl acetate	3.20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ethyl 2-methylbutyrate	3.69	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Hexenal	3.77	ND	ND	ND	3978	ND	ND	ND	ND	ND	ND
5-Hexen-1-ol	3.86	ND	3034	2254	3230	ND	ND	ND	ND	1326	7335
2-Hexen-1-ol	3.91	3648	4862	1569	7560	1587	5768	3713	512	ND	947
1-Hexanol	3.96	1943314	560860	481184	906110	541888	1232381	1191346	806504	471976	361606
1-Heptanol	4.11	ND	11833	ND	3876	10372	19876	17543	ND	ND	ND
2-Methylbutyl acetate	4.11	ND	10460	ND	ND	10018	16693	ND	ND	ND	ND
Not identified	4.24	7540	5921	6432	6720	6348	7602	7465	5892	4946	4553
Propyl butyrate	4.41	3286	ND	822	ND	ND	ND	ND	ND	ND	ND
Ethyl 3-hydroxybutyrate	4.98	ND	ND	ND	ND	ND	ND	ND	ND	24484	ND
2-Heptenal	5.38	9470	524	15677	954	ND	ND	1057	788	ND	470
Filbertone	5.63	2525	1507	2217	1855	2094	2239	1674	1378	1582	1378
1-Octen-3-one	5.70	3384	122	5733	ND	ND	202	481	275	ND	246
Sulcatone	5.83	16313	21003	6767	4804	5144	8896	8904	15682	10048	7014
Not identified	5.96	ND	ND	ND	ND	ND	168	ND	ND	ND	ND
Butyl butyrate	6.00	24798	ND	14179	2600	1240	9959	2768	5985	10902	ND
Ethyl hexanoate	6.06	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hexyl acetate	6.28	2232	ND	ND	ND	2034	2137	ND	2504	ND	ND
2-Ethyl-1-hexanol	6.54	4267	2230	3163	4815	2620	2632	1489	3155	1087	2331
Butyl 2-methylbutyrate	6.76	1330	1000	651	502	278	ND	ND	ND	ND	ND
Not identified	7.21	2017	860	1191	0	1072	917	745	956	634	792
1-Octanol	7.24	2920	ND	1076	12912	227	1675	336	2837	ND	1537
Not identified	7.77	ND	1552	3622	ND	ND	142	226	426	638	454
Nonanal	7.81	1357	705	1015	588	1171	832	717	633	ND	ND
Hexyl butyrate	9.16	18642	ND	969	11598	2695	ND	3369	1841	ND	2891
Hexyl 2-methylbutyrate	9.82	3526	6097	4263	3936	7857	ND	8839	ND	ND	ND
Not identified	9.84	6076	3098	5346	5993	4190	4023	4172	4101	2307	3319
1,3-Octanediol	10.13	10012	ND	2503	53426	ND	2804	18122	14613	5956	5192
Hexyl hexanoate	11.91	1324	ND	898	410	ND	ND	187	ND	ND	ND
Not identified	13.07	ND	ND	ND	980	929	ND	ND	ND	864	226
ᴧ-farnesene	13.48	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Not identified	13.66	ND	ND	ND	1601	ND	ND	1046	ND	ND	512

Source data from GS-MS chromatogram integrated by RTE integrator of the software used and rounded to integers are given.
Note: All results in Tab. 7. and Tab. 8 were calculated from unrounded source data.
Identification of apple samples is given in Tab. 1.
RT – retention time, ND – not detected, * – sum of coeluted peak areas.

Tab. 3. Results GC-MS analysis of apple varieties from organic production 2015.

Volatiles	RT [min^{-1}]	Apple samples									
		AB5	FB5	GB5	GoB5	IB5	MB5	OB5	RB5	SB5	TB5
		Peak area (abundance × second)									
Not identified	1.49	75577	46667	61842	39992	36873	33245	47441	43531	25864	59124
Not identified	1.58	17147	64128	39345	14422	44231	9304	21214	6446	10088	11537
Not identified	1.69	23967	14840	10190	39769	14773	8508	6321	11068	18997	9289
Not identified	1.78	22624	38834	50700	44257	36671	14144	38008	13316	19276	32100
1-Butanol	1.92	2715695	836555	587991	2004967	1010566	1529650	1700899	979300	1833447	387516
Pentanal	2.13	22962	16008	22230	35175	14958	18048	13406	11740	22044	16894
Bromodichloromethan	2.25	10741	13599	20005	10296	8721	4252	10305	6508	9936	4293
2-Methyl-1-butanol	2.44	987637	810840	2382682	168911	298150	34583	1260836	56791	48860	68135
1-Pentanol	2.68	63730	27287	40269	52418	13078	16690	45042	14079	24311	12654
Hexanal + ethyl butyrate*	3.06	23813	40587	30182	125589	19596	22604	18763	25572	122367	27606
Not identified	3.12	7301	12163	15019	ND	10926	10397	12441	5543	14022	5610
Butyl acetate	3.20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ethyl 2-methylbutyrate	3.69	ND	ND	ND	ND	ND	ND	2115	ND	ND	ND
2-Hexenal	3.77	ND	ND	ND	4606	ND	ND	ND	ND	ND	2917
5-Hexen-1-ol	3.86	ND	30110	1862	5848	ND	ND	ND	ND	2960	699
2-Hexen-1-ol	3.91	ND	1552	151	3728	1066	2885	1018	233	ND	2692
1-Hexanol	3.96	1134309	448533	654153	819483	524219	940423	747455	453466	566148	392481
1-Heptanol	4.11	1752	4878	ND	8647	15276	6453	14329	2309	ND	ND
2-Methylbutyl acetate	4.11	ND	6917	ND	7979	14246	ND	ND	ND	ND	ND
Not identified	4.24	6783	5221	6581	5896	5324	5617	6388	4991	4991	4465
Propyl butyrate	4.41	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ethyl 3-hydroxybutyrate	4.98	ND	ND	ND	ND	ND	ND	ND	ND	739	ND
2-Heptenal	5.38	3861	ND	14260	633	ND	273	ND	366	265	948
Filbertone	5.63	2482	1644	3206	2424	2590	1280	1835	2179	2516	787
1-Octen-3-one	5.70	1668	240	5581	578	ND	ND	69	257	361	352
Sulcatone	5.83	10411	7610	11616	7473	4335	7126	11325	3412	10301	19497
Not identified	5.96	ND	ND	ND	ND	ND	ND	923	ND	ND	ND
Butyl butyrate	6.00	3678	ND	ND	968	1782	743	7933	6769	6307	ND
Ethyl hexanoate	6.06	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hexyl acetate	6.28	2227	ND	ND	ND	3552	ND	ND	2302	ND	ND
2-Ethyl-1-hexanol	6.54	4971	1958	2910	3739	3588	1417	2717	4286	2438	1564
Butyl 2-methylbutyrate	6.76	91	ND	1042	245	224	ND	764	405	ND	ND
Not identified	7.21	1248	446	843	1102	1127	963	962	938	945	675
1-Octanol	7.24	3046	126	1907	9477	332	702	ND	1378	ND	1264
Not identified	7.77	ND	ND	4728	ND	ND	97	ND	112	ND	838
Nonanal	7.81	1385	948	890	1206	1270	707	1139	821	1035	ND
Hexyl butyrate	9.16	23305	ND	2372	4750	823	ND	10193	5974	2400	4794
Hexyl 2-methylbutyrate	9.82	8142	2324	8179	6282	6496	ND	14676	295	350	ND
Not identified	9.84	6255	3825	4858	6603	4151	3761	4822	4105	4058	3285
1,3-Octanediol	10.13	22459	232	2726	65061	1159	5215	17935	33348	43981	7356
Hexyl hexanoate	11.91	3448	ND	481	ND	ND	223	1038	732	ND	ND
Not identified	13.07	ND	ND	ND	ND	ND	ND	ND	ND	276	ND
ᴧ-farnesene	13.48	294	ND	ND	ND	ND	ND	ND	ND	ND	ND
Not identified	13.66	1687	ND	ND	ND	ND	ND	1514	171	1193	798

Source data from GS-MS chromatogram integrated by RTE integrator of the software used and rounded to integers are given.
Note: All results in Tab. 7. and Tab. 8 were calculated from unrounded source data.
Identification of apple samples is given in Tab. 1.
RT – retention time, ND – not detected, * – sum of coeluted peak areas.

Tab. 4. Results GC-MS analysis of apple varieties from integrated production 2016.

Volatile	RT [min⁻¹]	Apple samples						
		Al6	Fl6	Gol6	Il6	Ol6	Rl6	Tl6
		Peak area (abundance × second)						
Not identified	1.49	51506	41580	41001	22425	64126	28535	30136
Not identified	1.58	13172	43234	16069	32546	17140	ND	ND
Not identified	1.69	98582	182651	240941	132651	129408	187469	235511
Not identified	1.78	43561	54112	55830	34024	26146	16246	29803
1-Butanol	1.92	2421359	1586213	1998842	3340842	2585663	3066558	2957442
Pentanal	2.13	58528	31421	25998	30098	29559	27454	36594
Bromodichloromethan	2.25	22615	22817	13397	5917	9425	6083	3304
2-Methyl-1-butanol	2.44	505566	2302166	334743	1671054	1171717	228008	304708
1-Pentanol	2.68	93135	65923	34546	63257	100977	134323	127296
Hexanal + ethyl butyrate*	3.06	110345	194911	162853	164232	120001	418147	435546
Not identified	3.12	15833	21689	14169	3546	10398	ND	ND
Butyl acetate	3.20	ND	ND	96500	ND	ND	ND	ND
Ethyl 2-methylbutyrate	3.69	ND	ND	ND	ND	ND	ND	ND
2-Hexenal	3.77	3966	9811	12594	16387	9584	21366	22446
5-Hexen-1-ol	3.86	ND	3278	3857	ND	325	3479	2824
2-Hexen-1-ol	3.91	1935	3634	14144	3979	12488	14474	64430
1-Hexanol	3.96	1432777	1127203	2853960	2361694	3314834	7768505	5101573
1-Heptanol	4.11	ND	71459	72556	63674	85303	116957	97303
2-Methylbutyl acetate	4.11	ND	72093	64560	56153	94275	104850	107495
Not identified	4.24	10286	9684	11333	11031	13776	16510	13308
Propyl butyrate	4.41	14240	ND	ND	ND	7106	ND	ND
Ethyl 3-hydroxybutyrate	4.98	ND	ND	ND	ND	ND	ND	ND
2-Heptenal	5.38	61939	1471	13863	4339	2053	ND	5809
Filbertone	5.63	4461	4232	5122	2250	3489	2764	3619
1-Octen-3-one	5.70	23596	628	3425	1414	915	ND	1077
Sulcatone	5.83	25385	15065	18335	8914	20407	19029	55849
Not identified	5.96	2110	3059	4211	777	4633	5031	12264
Butyl butyrate	6.00	29221	2838	ND	3985	12266	2919	4180
Ethyl hexanoate	6.06	ND	ND	ND	ND	7565	ND	ND
Hexyl acetate	6.28	ND	ND	15326	ND	ND	ND	ND
2-Ethyl-1-hexanol	6.54	5672	4771	4535	2513	4201	4344	5024
Butyl 2-methylbutyrate	6.76	2496	6856	4625	2986	5058	ND	ND
Not identified	7.21	2778	1266	1284	1239	859	175	1426
1-Octanol	7.24	3849	1420	12607	528	4261	16708	2765
Not identified	7.77	96	3717	1606	754	1060	5601	ND
Nonanal	7.81	1856	1418	ND	1270	629	264	ND
Hexyl butyrate	9.16	33171	3913	3538	4010	14721	3571	4334
Hexyl 2-methylbutyrate	9.82	7295	19279	5450	14222	45510	1146	1400
Not identified	9.84	9327	7428	7091	6056	6792	6136	4338
1,3-Octanediol	10.13	2392	1407	15910	2618	14178	33535	18342
Hexyl hexanoate	11.91	2845	975	ND	ND	1228	1411	ND
Not identified	13.07	ND	ND	1093	ND	ND	ND	ND
ᴧ-farnesene	13.48	1139	ND	ND	ND	ND	ND	ND
Not identified	13.66	ND	65	ND	ND	1059	ND	ND

Source data from GS-MS chromatogram integrated by RTE integrator of the software used and rounded to integers are given.
Note: All results in Tab. 7. and Tab. 8 were calculated from unrounded source data.
Identification of apple samples is given in Tab. 1.
RT – retention time, ND – not detected, * – sum of coeluted peak areas.

Tab. 5. Results GC-MS analysis of apple varieties from organic production 2016.

Volatile	RT [min⁻¹]	Apple samples						
		AB6	FB6	GoB6	IB6	OB6	RB6	TB6
		Peak area (abundance × second)						
Not identified	1.49	29058	36386	30196	20037	32706	34549	26521
Not identified	1.58	18569	11311	11185	25397	13206	8775	ND
Not identified	1.69	151855	137770	102931	117365	141207	169585	113472
Not identified	1.78	31084	24528	44903	23098	28877	31579	38839
1-Butanol	1.92	3660193	2308295	2222277	3092926	1718528	2298660	2330572
Pentanal	2.13	49482	31430	27489	22535	40662	47205	29811
Bromodichloromethan	2.25	13332	11493	ND	ND	9868	12555	11276
2-Methyl-1-butanol	2.44	762129	101700	158244	1287073	1250300	723792	288424
1-Pentanol	2.68	144309	58493	65383	64657	79512	128936	97436
Hexanal + ethyl butyrate*	3.06	115317	68461	80408	139732	52518	383297	366186
Not identified	3.12	12449	10031	ND	5611	8754	9842	ND
Butyl acetate	3.20	ND	229091	ND	ND	ND	ND	ND
Ethyl 2-methylbutyrate	3.69	ND	ND	ND	ND	ND	2191	8462
2-Hexenal	3.77	2888	3878	19278	9167	1629	22599	60055
5-Hexen-1-ol	3.86	ND	344	11642	144	ND	456	20868
2-Hexen-1-ol	3.91	ND	2872	30310	11137	6462	30851	77351
1-Hexanol	3.96	3443570	2569446	1446986	2411665	1233724	3230860	3679013
1-Heptanol	4.11	ND	49726	2125	60840	31018	91222	225087
2-Methylbutyl acetate	4.11	ND	35031	ND	56276	26122	85921	193038
Not identified	4.24	19884	13812	8166	9528	9117	11520	17153
Propyl butyrate	4.41	22050	ND	ND	ND	ND	ND	ND
Ethyl 3-hydroxybutyrate	4.98	ND	ND	ND	ND	ND	ND	ND
2-Heptenal	5.38	24144	1050	1553	1176	4018	4315	ND
Filbertone	5.63	5092	2848	256	2271	2272	4043	4408
1-Octen-3-one	5.70	7085	ND	354	301	771	931	ND
Sulcatone	5.83	29340	8281	3955	8411	5600	25638	126337
Not identified	5.96	2348	2760	ND	557	819	4549	28199
Butyl butyrate	6.00	34149	5565	ND	2759	737	4429	10005
Ethyl hexanoate	6.06	ND	ND	ND	ND	1282	ND	ND
Hexyl acetate	6.28	ND	81034	ND	ND	ND	ND	ND
2-Ethyl-1-hexanol	6.54	6118	4220	ND	1830	2461	3239	5671
Butyl 2-methylbutyrate	6.76	3997	3967	539	1242	865	ND	583
Not identified	7.21	1557	1430	709	531	559	1253	197
1-Octanol	7.24	11573	1204	2842	ND	ND	1458	23682
Not identified	7.77	1335	ND	ND	ND	ND	647	3227
Nonanal	7.81	691	1497	821	820	1048	1109	ND
Hexyl butyrate	9.16	49549	6317	6537	2150	1309	3847	16073
Hexyl 2-methylbutyrate	9.82	9968	1161	1577	12824	5252	1595	7475
Not identified	9.84	7687	6326	6856	5436	6038	4420	5666
1,3-Octanediol	10.13	12291	4222	27786	5124	ND	11094	33761
Hexyl hexanoate	11.91	5198	1475	ND	ND	ND	ND	2527
Not identified	13.07	ND	ND	2736	ND	ND	ND	ND
Λ-farnesene	13.48	466	ND	442	ND	ND	ND	2658
Not identified	13.66	ND	194	696	ND	ND	ND	5297

Source data from GS-MS chromatogram integrated by RTE integrator of the software used and rounded to integers are given.
Note: All results in Tab. 7. and Tab. 8 were calculated from unrounded source data.
Identification of apple samples is given in Tab. 1.
RT – retention time, ND – not detected, * – sum of coeluted peak areas.

resented the reason for the dominant influence of the factor year. Therefore, it could be assumed that it was the climatic conditions that most likely strongly influenced the results.

If we compare the results of soluble solids in 2015 and 2016, in 2015 most varieties showed higher values than their analogues in 2016. (Tab. 6). According to the information from the Czech Hydrometeorological Institute and the Ministry of Environement of the Czech Republic, the year 2015 belonged to the year with an extreme summer drought, exceptionally above normal year temperature and year rainfall strongly below normal, which could be the reason for the higher content of soluble solids in apples harvested in 2015 [18, 27, 28]. The highest values of soluble solids indicating higher expected sweetness were found in Angold, Florina and Goldstar varieties organically grown in 2015. However, Ontario grown in integrated system in 2015 was significant by higher soluble solids as well. The differences between apples grown in different farming systems were not significant, however, it was observed that the majority of organically produced apples had higher values of soluble solids than the same cultivars from integrated production (except for Ontario and Rubin 2015 or Florina, Idared and Ontario 2016, Tab. 6). The values of soluble solids determined in this study were, in the majority of cases, in accordance with the range of the values reported for the same cultivars in the literature [29–34]. Regarding the results of titratable acidity (Tab. 6), they could not be used to discriminate samples from organic and integrated production, as the higher acidity values could not be attributed to any of these farming systems. Comparing the individual cultivars, cultivar Topaz (varieties TB6 and TI5), was characteristic by higher acidity, which was similar to data reported in literature [29, 32]. Furthermore, the cultivars Melodie (varieties MB5 and MI5) and Ontario (all samples) had high levels of acidity as well. The same conclusions were gained by evaluating the results on soluble solids and acidity by ANOVA (Tab. 7). The determined acidity values of individual cultivars (Tab. 6) corresponded to the range of the values presented for tested cultivars in literature [29, 31–34]. It can therefore be assumed that the affiliation of apple samples to particular cultivars could predestine their acidity.

The contents of 45 dominant volatile compounds having at least in one variety the peak area larger than 1 800 (expressed as abundance × second, Tab. 2–5) were selected for statistical evaluation. The results on 14 of them, typical apple volatiles widely covered in literature

Tab. 6. Titratable acidity and soluble solids determined in various apple varieties.

Apple samples	Titratable acidity [g·kg⁻¹]	Soluble solids [%]
AB6	5.38 ± 0.11	12.8 ± 0.1
AI6	4.24 ± 0.32	10.1 ± 0.1
FB6	8.23 ± 0.03	10.5 ± 0.0
FI6	4.32 ± 0.10	12.2 ± 0.0
GoB6	6.23 ± 0.33	13.2 ± 0.1
GoI6	6.19 ± 0.24	10.1 ± 0.0
IB6	6.60 ± 0.03	11.1 ± 0.0
II6	5.91 ± 0.03	12.6 ± 0.0
OB6	9.33 ± 0.66	9.2 ± 0.0
OI6	9.78 ± 0.29	10.3 ± 0.0
RB6	6.78 ± 0.25	14.2 ± 0.0
RI6	3.87 ± 0.13	11.8 ± 0.0
TB6	9.05 ± 0.01	13.5 ± 0.4
TI6	4.66 ± 0.16	11.4 ± 0.0
AB5	6.55 ± 0.26	15.0 ±0.2
AI5	6.64 ± 0.29	11.9 ± 0.1
FB5	5.32 ± 0.28	15.0 ± 0.1
FI5	6.56 ± 0.11	14.5 ± 0.1
GB5	5.89 ± 0.02	12.1 ± 0.0
GI5	5.72 ± 0.02	10.5 ± 0.2
GoB5	5.87 ± 0.03	16.1 ± 0.0
GoI5	4.15 ± 0.11	11.5 ± 0.1
IB5	5.00 ± 0.06	10.5 ± 0.0
II5	5.21 ± 0.06	9.7 ± 0.1
MB5	8.76 ± 0.01	12.0 ± 0.1
MI5	8.29 ± 0.00	11.3 ± 0.1
OB5	9.46 ± 0.23	13.3 ± 0.1
OI5	10.33 ± 0.39	16.1 ± 0.1
RB5	5.18 ± 0.01	11.1 ± 0.0
RI5	5.31 ± 0.10	13.9 ± 0.1
SB5	4.26 ± 0.41	12.8 ± 0.1
SI5	5.59 ± 0.30	11.2 ± 0.1
TI5	8.39 ± 0.20	11.0 ± 0.0
TB5	na	na

Values represent mean ± standard deviation calculated for each apple variety from its two samples. Titratable acidity is expressed as grams of malic acid per kilogram of the sample. Soluble solids are expressed as percentage of soluble solids in the sample.
Identification of apple samples is given in Tab. 1.
na – not available

regarding their dominance or aroma contribution [9, 14, 15, 26, 35–37], including those mentioned in the introduction because of changes conditioned by farming system [9, 10], were evaluated by univariate ANOVA (Tab. 7), involving samples from both years. The table shows the influence of type of production, cultivar and harvest year on individual volatile substances, acidity and soluble solids. The significant influence of the year is evident. As can be seen from the last

Tab. 7. Results of ANOVA.

Parameter	*p*-value		
	Cultivar (9 degrees of freedom)	Type of production (1 degree of freedom)	Year of harvest (1 degree of freedom)
1-Butanol	0.183	0.983	0.001*
2-Methyl-1-butanol	0.001*	0.594	0.177
Hexanal with ethyl butyrate	0.434	0.633	0.002*
Butyl acetate	0.785	0.604	0.215
Ethyl 2-methylbutyrate	0.813	0.229	0.365
2-Hexenal	0.398	0.661	0.003*
2-Hexen-1-ol	0.339	0.729	0.023*
Hexanol	0.756	0.374	0.001*
2-Methylbutyl acetate	0.520	0.567	0.002*
Butyl butyrate	0.019*	0.236	0.144
Ethyl hexanoate	0.563	0.429	0.271
Hexyl acetate	0.803	0.467	0.352
Hexyl butyrate	0.002*	0.457	0.107
Acidity	0.007*	0.119	0.814
Soluble solids	0.778	0.198	0.071

Contribution of factors cultivar, type of production and year of harvest to the importance of 14 selected volatiles, acidity and soluble solids.
* – *p*-value < 0,05 means great importance.
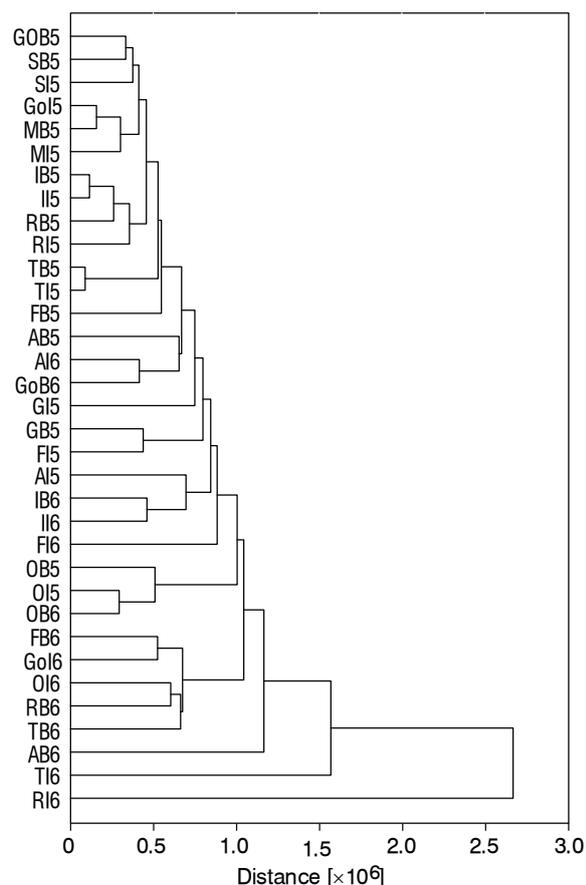
Fig. 1. Cluster analysis of the results for 45 main volatiles determined in apples.

Identification of apple samples is given in Tab. 1.

column corresponding to the year of the harvest, the value of 1–*p* (so called level of importance, gained after the calculation from the *p*-value in Tab. 7) tended to be close to 100 % for most of the volatiles contrary to the 1–*p* values calculated for identical substances in relation to other two factors. Especially for 1-butanol, hexanal with ethyl butyrate, 2-hexenal, 2-hexen-1-ol, hexanol and 2-methylbutyl acetate, year of harvest had a great importance (*p* < 0.05). The influence of cultivar was noticeable in ANOVA results on 2-methyl-1-butanol, butyl butyrate, hexyl butyrate and acidity (*p* < 0.05).

In cluster analysis of 45 dominant volatiles, apple varieties were grouped on the basis of their similarity (Fig. 1). The separation according to harvest year was obvious (year 2015 dominated on the left, year 2016 on the right), however, no differentiation with production type was noticeable. These outcomes proved the strong impact of the factor year and therefore also of the related climatic conditions. Simultaneously, similarity of the same cultivars is conspicuous due to their close distances (TI5 and TB5, II5 and IB5, OB6 and OI5). Thus, similarly as in the case of ANOVA, more significant contribution of the factors year and cultivar to differentiation of apples was observed than that of the farming system.

To provide further information, results on 14 typical volatiles judged by ANOVA were evaluated by discriminant analysis of all tested

Tab. 8. Results of discriminant analysis.

Variable	Wilks' λ	Partial λ	p-value
1-Butanol	0.06	0.93	0.71
2-Methyl-1-butanol	0.06	0.88	0.51
Hexanal with ethyl butyrate	0.06	0.94	0.76
Butyl acetate	0.07	0.80	0.26
Ethyl-2-methyl butyrate	0.12*	0.45*	< 0.01*
2-Hexenal	0.08	0.69	0.07
2-Hexen-1-ol	0.06	0.91	0.61
1-Hexanol	0.06	0.93	0.73
2-Methylbutyl acetate	0.07	0.79	0.23
Butyl butyrate	0.07	0.71	0.10
Ethyl hexanoate	0.05	0.96	0.88
Hexyl acetate	0.06	0.83	0.32
Hexyl butyrate	0.06	0.88	0.51

Contribution of 14 selected volatiles to discrimination between apples of organic production 2015, organic production 2016, integrated production 2015 and integrated production 2016.
Wilks' λ – statistical significance of the discriminatory power for the overall model that will result after removing the respective variable from the model, partial λ – statistical significance of the discriminatory power for the unique contribution of the respective variable to the discrimination between groups (0 – perfect discrimination; 1 – no discrimination).
* – values signalizing biggest contribution of ethyl-2-methylbutyrate to discrimination between groups.

apples (Tab. 8, Fig. 2). Data were assigned to four groups with diverse combinations of two factors, year of harvest and production type, according to their affiliation to these groups. Even in this case, the separation of harvest years 2015 and 2016 was unambiguous (Fig. 2). At the same time, however, there was no visible differentiation between apples from organic and integrated production in 2015. The impact of climatic conditions could contribute to the mutually intertwined results. For 2016, a shift towards differentiation of organic and integrated production was observable. The substance ethyl-2-methyl butyrate (E2MB) significantly contributed to the discrimination between the groups according to p-value smaller than 0.05 (Tab. 8). The lowest value of partial lambda (partial λ) for E2MB, compared to the other values in Tab. 8, indicated this compound's biggest contribution to discrimination. The second most contributing was 2-hexenal with the second lowest value. The biggest contribution of E2MB was confirmed by the highest value of Wilks' λ representing the value after excluding the variable E2MB from the model. Such exclusion was not advantageous for the purpose of discrimination between groups, which was why the value of Wilks' λ was the highest for this compound. Reversely, its inclusion into the model was important for discrimination because of the lowest E2MB's value of partial λ. More specific conclusions follow from visual assessment of the results. The result observed for E2MB was

the same as statistical, regarding the substance's presence only in organically grown apples, as its contribution to discrimination between production systems was clear. In case of the substances hexyl butyrate and 1,3-octanediol, the most of the va-
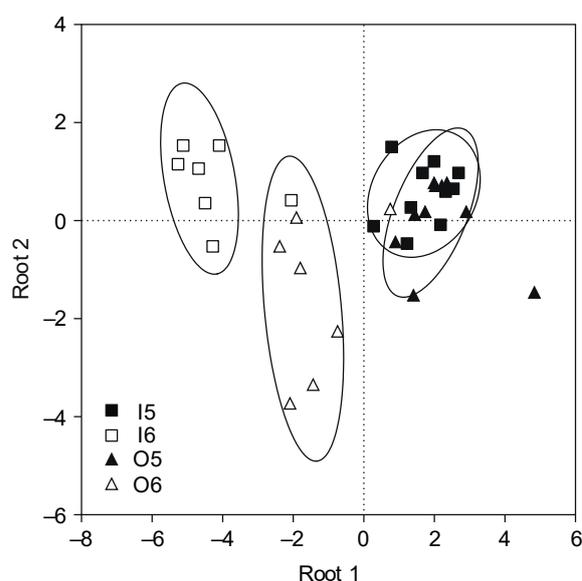
Fig. 2. Discriminant analysis of the results for 14 selected apple volatiles.

I5 – integrated production 2015, I6 – integrated production 2016, O5 – organic production 2015, O6 – organic production 2016.

rieties of organically grown apples provided higher values than it was observed for their analogues from integrated production (Tab. 3–5). Similarly in case of 2-heptenal, most of the varieties of integrated production reached higher values than their organically grown analogues. Anyhow, inclusion of 1,3-octanediol and 2-heptenal to discriminant analysis and ANOVA did not bring dramatic changes to final judgements. Discriminant analysis identified E2MB again as the only volatile with $p < 0{,}05$ and 2-hexenal as a second contributing to discrimination, 2-heptenal was third and was followed by 2-methylbutyl acetate, 1,3-octanediol and butylbutyrate. Inclusion of 1,3- octanediol and 2-heptenal to ANOVA did not show special significance, their *p*-values for the factor type of production (0.290, 0.304) were higher than those of ethyl 2-methylbutyrate and butyl butyrate in Tab. 7. By closer focus on the results of each year separately (Tab. 3–5), the reason for the shift towards differentiation of organic and integrated production in 2016 was clearer. Many volatiles of 2016 apple varieties grown in integrated farming conditions were conspicious by their higher values compared to the values of their organic analogues. In contrast, for 2015, only 2-hexen-1-ol was conspicious by higher values of one production system (in this case again integrated) for most of the results. Namely, in 2016, hexanal and ethyl butyrate, 2-methylbutyl acetate, butyl-2-methyl butyrate, 1-heptanol, 1-octen-3-one, 2-ethyl-1-hexanol and 1-octanol reached higher values for most of integrated varieties compared to their organic analogues. Exceptions were observed mainly in case of organically grown Angold and Topaz cultivars (varieties AB6 and TB6), for which higher values were found for majority of the eight named volatiles, while their analogues grown in integrated system (AI6 and TI6) had lower values. The reason for higher values in organic conditions in case of Topaz cultivar might be connected to specifity of this cultivar, noticed in context of higher acidity values observed in literature [29, 32] as well as to the year conditions. The results on 2-hexen-1-ol, previously mentioned as conspicious for 2015 varieties, contribute to the idea of Topaz cultivar specifity, as this cultivar was the only one among 2015 apples noticed as having higher value of 2-hexen-1-ol in organically grown variety (TB5). However, for the Angold cultivar, higher values for volatiles were observed only for variety AB6 organically grown in 2016, which indicated rather influence of the year than the cultivar specifity. Simultaneously in 2016, higher values for volatiles in apples of integrated production contributed to the observed differentiation of integrated and organic production systems. It seems that both agricultural systems in 2016 strengthened their features.

It can be supposed that differentiation of the systems was not caused by the diverse agricultural approach in individual years. As it was described in material section, agricultural methods were kept the same in both years 2015 and 2016. The year period seemed to be a reason for the strengthening of agriculture system features in the second year. It is typical that soil becomes established and humus and permanent soil fertility in ecological and integrated orchards is created during several years [38–40]. Simultaneously, the organic crops or their quality differ significantly from conventional crops or differ from crops of integrated farming depending on the soil development and environmental impacts including climate [41, 42]. Regarding the statistical data, the overall correctness of classification for discriminant analysis model was 76 %, for 2016 varieties 85 %. Inclusion of soluble solids and acidity results to cluster analysis and discriminant analysis did not lead to a change in the overall conclusion about the influence of discussed factors regarding the obvious similarity of resulting forms of figures with Fig. 1 and Fig. 2, therefore it is not discussed in more detail.

Considering all results, different conditions in individual years, induced by climate, showed stronger impact on volatile metabolites and qualitative parametres of apples than the farming system in which they were grown. Simultaneously, based on ANOVA and cluster analysis, the similar judgement can be done about the factor cultivar. These findings are similar to those of LE BOURVELLEC et al. [11], where cultivar and year conditions were the main factors effecting primary and secondary metabolites in apples, while the management system had little influence on the fruit composition. At the same time, however, it is necessary to take into consideration the limited possibilities of our experience resulting from to the two years experiment, short duration to observe trends of several years.

CONCLUSION

Whether specific volatile substances could help to distinguish between fruits from different farming conditions remains a question. Any differences between farming systems were noticable in apples harvested in 2015 based on statistically processed data. However, a shift towards differentiation of apples from organic and integrated production was observed for 2016. More statistically significant differences were observed for parametres of

apples harvested in different years, or between individual cultivars, than between farming systems. Namely, in case of volatiles, ANOVA showed that the harvest year was the factor causing the most of significant differences between them, and the factor cultivar was the second most contributing. Similarly, correlations between volatiles in individual years were observed by discriminant analysis and cluster analysis. Moreover, cluster analysis revealed correlations between volatiles of individual cultivars. In case of soluble solids and acidity results, ANOVA analogically showed the harvest year as the factor having the biggest importance on differences between soluble solids values and cultivar as the factor having the biggest importance on differences between acidity results. Dry climatic conditions in 2015 with extreme summer drought may have contributed to higher soluble solids values determined in apples harvested that year.

Supplementary data

Supplementary data related to this article can be found at http://www.vup.sk/download.php?bulID=2142.

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