

Aromatic compound in different peach cultivars and effect of preservatives on the final aroma of cooked fruits

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Abstract

Four yellow-fleshed peach cultivars („Royal Glory“, „Redhaven“, „Maria Marta“ and „Norman“) were used during two-year period in this study. The characterization of aromatic constituents of investigated cultivars was done using headspace solid phase micro-extraction (HS-SPME). The intention was to make implicit discrimination between cultivars by analysis of components present in all cultivars during two-year period. Also, the impact of added preservatives (Na-benzoate and citric acid) on the final aroma of cooked peaches was studied. The cultivars' differences and the impact of preservatives (Na-benzoate and citric acid) were statistically evaluated. Multiple discriminant analysis of peaches' aromatic profile was used to segregate investigated peach cultivars. Although they were very similar, the cultivars were segregated by two discriminant functions, function 1 (which accounted for 56.9% of this peach model) and function 2 (31.7%). The use of preservatives had also an important impact on the aromatic profile of cooked peaches. The statistical analysis indicated that from 57 identified volatiles, 40 volatiles showed statistically significant difference regarding the way of preservation. The main negative impact had Na-benzoate compared to control or samples preserved with citric acid.

Keywords: peach aroma, peach volatile compounds, discriminant analysis, food preservatives, solid phase micro-extraction.

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Peaches and apricots are among the most delectable fruits available and they are cultivated for family consumption or for sale on local markets. The tree ripe fruit can reach the consumer only in local markets, while regarding to market needs they are mostly picked immature. An earlier survey conducted by UC Davis researchers indicated that the main reasons why consumers do not eat more stone fruits are their lack of taste, failure to ripen, fruit is hard and mealy [1]. Peaches, nectarines, plums and apricots, are all members of the *Prunus* genus and therefore are closely related. They commonly are referred to as “stone fruits” because the seed is very large and hard. Thus, it is useful to determine the main volatilities which correspond directly to different maturity stage, accumulated either in the case when fruits were exposed to injuries or they could be indicative for rotten fruit. There is a lack of research about peach aroma development and aroma stability, as well as a suitable

classification among cultivars and their label on the market.

The evaluation of the flavour ranged on scales is usually done by panellists, and to all advanced research-oriented growers the sensory evaluation is certainly helpful [2–4]. Careful evaluation of single aromatic compound and a group of compounds that have a specific impact on the overall fruit aroma are certainly useful. Within components which defining peach aroma, we recognize (*E*)-2-hexenol and (*E*)-2-hexenal, as representatives of “green aroma”. On the other hand, as representatives of fruity, ripe aroma, γ -decalactone and γ -dodecalactone are detected [5]. During ripening the amount of C6 compounds decreases, while the concentration of lactones increases [6,7]. Peach ripening and the development of typical peach aroma are strongly dependent on the content of the lasts (δ - and γ -decalactones) [8]. Regarding to this remark, they are commonly used as markers in electronic transducers (e-nose) in fruit cool chambers [9–11]. Comparison of fresh and canned Halford peaches resulted in differences more due to the relative concentration of esters and monoterpenes than to γ -lactones [12]. Another study of chemical composition evaluation, firmness and variations in the colours of the

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fresh and canned peaches showed that picking the fruit more matured resulted in higher colour and flavour quality of products [13].

The contents of organic acids and sugars in foods and their relative ratio can provide precious information on food wholesomeness or to show how to optimize some selected technological processes [14]. Furthermore, the nature and concentration of the organic acids in fruits influence on their sensory characteristics, particularly on flavour, colour and aroma, and stability of fruit juices [15]. Sucrose, glucose, fructose and sorbitol are the main sugars or sugar alcohols encountered in the fruit of most plants from Rosaceae family. The edible quality of peaches (*Prunus persica* L. Batsch) depends to a great extent on their sweetness, which is related to sugar composition [16].

The relation between peach aromatic compounds, their evolution or/and masking effects on the one side and impact of temperature and preservatives on the other has not been much experimentally studied. Cooking during simultaneous steam distillation has an impact on the aromatic profile of the peaches generated an overall more intensive flavour extract [17]. Generally, in the industrial conditions, the high temperatures and times are applied, but they are specific for each procedure and purpose. There is a huge group of chemical preservatives too, which could be used to preserve the storage stability of juices, nectars and jams [18–20]. Citric acid has been widely used as an effective preservative due to its ability to reduce the superficial pH of cut fruits such as orange [21], peach, apricot and kiwifruit [22]. The use of another preservative sodium benzoate is highly disputable, since its application is connected with side health effects including increased hyperactivity [23], and asthma seemingly due to food allergy [24]. In the animal study it was shown that chronic exposure and use of benzoic acid and sodium benzoate can lead to reduced feed intake and reduced growth [25]. Moreover, very high doses of benzoic acid cause adverse effects such as metabolic acidosis, convulsions and hyperpnoea [26]. The EU directives on food additives other than colours and sweeteners (94/35/EC, 94/36/EC and 95/2/EC) regulate the use of permitted food additives. In subsidiary legislation 449.48 a limited number of additives are permitted for use in foodstuffs. For jams and similar products, the citric acid (E330) is allowed in *quantum satis* (as much as suffices) maximum level, whereas the maximum level for fruit juices and nectars is 5 g/L. Conditionally permitted preservative, such as sodium benzoate (Na benzoate) (E211) and benzoic acid, is allowed from 150 to 500 mg/kg. On the other hand, the US Code of Federal regulations (number 184.1021) regulates the use of benzoic acid in a concentration of 0.1% in food.

The principal component analysis (PCA) is a simple, chemometric, linear, unsupervised, and pattern recognition technique used for analysing, classifying, and reducing the dimensionality of numerical datasets in a multivariate problem [27]. Multivariate statistical analyses, employing PCA, were effective in describing chemical (pH, sugars and acids) and sensory (sweetness, fruity, sourness) variables in order to characterize peach quality [28,29]. The PCA was used in order to discriminate the optimum maturity date for harvesting peaches (model cultivar “Royal Glory”) and nectarines (“Big Top”) [30]. Also, peach juices (cv. “Redhaven”, “Suncrest” and “Maria Marta”) were clearly distinguished by PCA, with the most relevant variables, such as glucose, fructose, sorbitol, malic acid and colour parameters [31]. Further, PCA was used to segregate cultivars into different organoleptic groups [28], and 4 typical volatility groups (the groups with higher content of lactones, terpenes, esters and linalool) [32]. Discriminative analysis (DA), on the other hand, is one of the most widely used classification procedure. This method maximizes the variance between categories and minimizes the variance within specific category. DA was efficiently applied in order to discriminate apricot, pear and peach nectars according to their production, organic, conventional or/and flavoured characteristics [33]. According to our knowledge, discrimination of cultivars using the same volatile substances present in all examined cultivars, by exclusion of those which are typical only for one cultivar that has not yet been fully investigated.

The aim of the present work was to create the volatility profile, and to determine acids and sugars’ content of four peach cultivars, “Royal Glory”, “Redhaven”, “Maria Marta” and “Norman”. We also tried to make discrimination of peach cultivars by the analysis of commonly found substances present in all cultivars under investigations, with the simultaneous exclusion of those, which are typical only for one cultivar, during two seasons (2009 and 2010). Further, the impact of added preservatives (chelating agents, citric acid and sodium-benzoate) on volatiles and their evolution and/or masking effect in cooked peaches were studied.

EXPERIMENTAL PROCEDURE

Plant material and sampling

The trial was performed during 2009 and 2010 at the Fruit Growing Centre of Bilje near Nova Gorica (N 45° 53.528'; E 13° 38.606'), Slovenia. Four yellow-fleshed peach cultivars: “Royal Glory”, “Redhaven”, “Maria Marta” and “Norman” were sampled at three stages of ripening: pre-technological maturity, commercially ripe and tree-ripe in July 2009 (monthly rainfall 122.8 L/m²) and July 2010 (monthly rainfall

200.3 L/m²). The trees were irrigated and hail net was used to protect them from bad weather. The orchard was managed according to standard integrated pest management plan approved in 2007 by the Ministry of Agriculture, Forestry and Food of Slovenia. Chosen cultivars are freestone types, they have melting flesh that becomes soft and juicy as it ripens. This type of peaches usually is preferred for eating fresh or for processing, and commonly grown in southern EU. Approximately 30 undamaged peaches (~4–5 kg) randomly sampled (east, west, north, south, top and bottom) from 5–7 trees of each cultivar (rootstocks were planted in a permanent place in spring 2005 and grafted with cultivars in August 2005) were harvested in the period ranged from 7 to 10 days in seasons 2009 and 2010. From each fruit three longitudinal slices (from stem end to calyx-end) are taken. Then the slices were put in the blender. The blender was equipped with a tube, through which a gentle stream of nitrogen was passing in order to prevent oxidation during mixing. The peach pulp was then immediately analysed, a part of it was immersed into liquid nitrogen (for volatile analysis) and the other part was transferred into the freezer at -16°C (for acids and sugars analysis).

Analytical procedure

Chemicals

Chemicals: 1-octanol (the internal standard), lactones components (γ -hexalactone, γ -heptalactone, γ -octalactone, γ -nonalactone, γ -decalactone and δ -decalactone), terpenes (α -terpineol, β -citronell, geraniol, and nerol), (*E*)-2-hexenal, (*Z*)-3-hexenol and 2-nonenol were purchased from Sigma Aldrich, Germany. Further, 1-hexanol, hexanal, 1-heptanol, linalool, octanal, (*E*)-2-hexenyl acetate, *n*-hexyl acetate and 2-nonenol were obtained from Alfa Aesar, Germany. Most standards were analytical grade or at least of 95% purity. Citric acid was obtained from Sigma Aldrich, and the analytical grade was >99.7%. Sodium benzoate was purchased from Alfa Aesar and was 99% pure.

Analysis of volatiles

20 g (in flask) of peach pulp (frozen in liquid nitrogen) was spiked with internal standard 1-octanol (0.23 mg/kg final concentration in pulp) and placed into the gas-tight 50 mL artisan made single-necked round-bottom flask. The flask was then put in the magnetic stirrer water bath (Ecorototherm from Dinkelberg analytics GmbH) at 40 °C for 10 min for thermal equilibration, and left for 20 min for solid phase micro-extraction (SPME). SPME device (manual holder, Supelco, Bellefonte, PA, USA) with a 10 mm fibre coated with 50/30 μ m carboxen/polydimethylsiloxane/divinylbenzene – DVB/CAR/PDMS, was used for the extraction. After extraction, the SPME device was introduced in a gas chromatograph with mass selective detector (GC–

–MS – Agilent 6890 Series GC System with Agilent 5973 mass selective detector) in the splitless injector 270 °C for 10 min. Daily, prior analysis, the fibre was conditioned and activated by inserting it into the GC injector at 270 °C for 30 min. The volatiles were separated on Rtx-20 column (60 m, 0.25 mmID, 1 μ m, Restek, USA). The temperature program was as follows: initial temperature 50 °C (2 min) – 10 °C min⁻¹ – 150 °C (for 3 min) – 10 °C min⁻¹ – 250 °C (for 5 min). Total run time was 30 min. The mass spectrometer was operated in the electron ionisation mode at a voltage of 70 eV, the temperature of the MS Quad was set at 150 °C and the ion source at 230 °C. Compounds were identified on the basis of their retention times (comparison with standards) and spectra using the searchable EI-MS spectra library (NIST02). The peak areas needed for qualification and quantification were measured either in TIC chromatogram or in an extracted ion chromatogram in the case of co-elution with other compounds. The average RSD of the method applied was 13%.

Analysis of sugars and acids

10 g of peach pulp was diluted with 40 mL of deionised water. In order to achieve the best resolution and precision, two procedures were compared, direct injection and sample clean-up with Bond Elute® Sax cartridge (Varian, Harbor City, CA). Diluted samples were filtered through a 0.45 μ m cellulose acetate membrane before direct injection.

In comparison with this simplified procedure, pre-treatment with Sax cartridge, following the procedure (with some modifications) according to Chinnici *et al.* was applied. The solid phase extraction (SPE) employing Sax cartridges offered the separation of neutral compounds (*e.g.*, sugars and alcohols) from the acidic ones. The cartridge (3 mL/500 mg) was preliminarily conditioned with methanol (3 mL) and deionised water (3 mL). Then the diluted sample (5 mL, corrected to pH 7.0) was slowly passed through the cartridge (less than 0.2 mL/min). The neutral compounds were not retained, whereas the acidic ones were afterwards eluted with 2.5 mL of acid and washed with 2.5 mL of deionised water. The two fractions (the first containing neutral compounds – sugars and sorbitol and the second containing acids) were injected in the HPLC apparatus. The HPLC system (Agilent 1100) was equipped with quaternary pump (G1311A), a variable wavelength detector (G1314A) set at 210 nm (for the analysis of organic acids) connected in series with a refractive index detector (G1362A) (for sugar analysis), and an injection valve fitted with a 20 μ L loop. The samples were isocratically separated at 0.6 mL/min, using a Bio-Rad Aminex HPX 87H column (300 mm \times 7.8 mm i.d.) at 30 °C. Diluted sulphuric acid (0.005 M) was used as a mobile phase. Quantification was carried out using the external standard method, with the $R^2 \geq 0.9999$ in all cases.

Addition of preservatives

According to EU directives on food additives other than colours and sweeteners (94/35/ES, 94/36/ES, 95/2/ES) and the US Code of Federal regulations (number 184.1021), which regulates the use of benzoic acid and its salt, the added concentration of citric acid and sodium benzoate was in all cases of 0.1% of peach pulp weight. Thus, 20 g of peach pulp (individually made of four yellow-fleshed peach cultivars: “Royal Glory”, “Redhaven”, “Maria Marta” and “Norman” sampled in year 2010 frozen in liquid nitrogen) was spiked with internal standard 1-octanol (0.23 mg/kg final concentration in pulp) and 20 mg of either citric, or sodium benzoate and placed into the flask, capped and put in the water bath (Julabo TWB22) at 95 °C for 30 min for imitating the cooking process. Then the flask was transferred into the magnetic stirrer water bath (Ecorotherm from Dinkelberg analytics GmbH) at 40 °C for 10 min for thermal equilibration, and left for 20 min for solid phase micro-extraction. The procedure followed steps was previously described under *Analysis of volatiles* Section.

Data treatment

Descriptive statistics, such as arithmetic mean and range, were used to describe the main features (comparison) of four yellow-fleshed peach cultivars. Analysis of variance was used to determine the differences between individual cultivars, as well as the impact of different preservatives on volatile profile. For this purpose two factorial ANOVA was carried out to test the main effect of cultivars (C), preservative method (P) and their interaction. Tukey's multiple comparison tests were performed to determine the differences between group means.

Forward stepwise discriminant analysis with Wilks' lambda method was used to determine the differences between the individual cultivars. By using a stepwise selection procedure, only the most significant discriminant variable was identified. The scatter plot of discriminant scores corresponding to each sample (regardless of the season and maturity) in the multivariate space defined by the first two discriminant functions was introduced to present a multivariate volatile profile variation between examined groups. All computations were carried out with SPSS Statistics 18.0 and Statistica for Windows software.

RESULTS AND DISCUSSION

Differences in peach volatiles regarding the season and variety

The analysis of volatile components in investigating cultivars during seasons 2009 (monthly rainfall 122.8 L/m²) and 2010 (monthly rainfall 200.3 L/m²) was pre-

sented in Table 1. On the basis of gained results we found that the influence of external factors, like monthly rainfall on the overall amount of volatiles identified in the specific cultivar, is very important. The influence of seasonal differences was confirmed for the particular components in all 4 cultivars, and that results are pointed out (marked **bold**). In the group of alcohols, the seasonal difference was observed in the case of 1-nonanol, which was present in 3 cultivars only in year 2010 (in Norman it was absent both years), whereas in the year 2009 was not detected. Higher concentrations of hexanal, (*E*)-2-hexane, heptanes (except for Redhaven) and 2,5-furandicarboxaldehyde were found in all 4 cultivars in the year 2009. The C6 compounds (hexanal and (*E*)-2-hexenal) are the representatives of peach green aroma and are directly proportional to the stage of peach ripeness [34–36]. Besides that, aldehydes are for most varieties the most abundant compounds. Esters, which were found to be susceptible to monthly rainfall, were ethyl acetate, pentyl acetate, 3-hexenyl acetate, ethyl-2(5*H*)-furanone, octyl acetate and all identified lactones. However, lactones are known to increase with maturity [37,38].

The season had the most pronounced effect in the group of terpenes, since their concentration was significantly higher in all cases in season 2009. In fact, obtained results are in accordance with the observation, that terpenes synthesis increases considerably at high temperatures resulting in high concentrations in tissues, which led to the hypothesis that emitted terpenes may protect plants from high temperature [39]. Similarly, it was found that water deficit increased the concentrations of several individual monoterpenes and resin acids in conifers [40]. There are some assumptions that carbon fixed in photosynthesis could be used for the formation of secondary metabolites. However, our study is the first report indicating the negative effect of rainfall on the concentration of terpenes in fruit.

On the basis of the obtained results from Table 1, beside seasonal also cultivars' differences could be observed. (*E*)-2-nonenal (tallowy, fatty) and 2-methylhexyl propanoate (fruity, green, ripe, sweet) were detected only in “Royal Glory”, (*Z*)-2-heptenal (fatty), (*E*)-2-octenal (green, fatty) were found in “Maria Marta” and “Norman”, 3(2*H*)-benzofuranone, octyl acetate (orange) and (*E*)-2-hexenyl hexanoate (green, fruity, cheesy) in “Royal Glory” and “Redhaven” and *cis*-geraniol (rose-like odor) was found only in “Redhaven”.

Discriminant analysis

Considering we followed the evolution of peach volatiles during 2009 and 2010 at three stages of ripening: pre-technological maturity, commercially ripe and tree-ripe on four yellow-fleshed peach cultivars: “Royal Glory”, “Redhaven”, “Maria Marta” and “Nor-

Table 1. Minimum-maximum range and arithmetic means of identified volatiles; main seasonal differences are marked **bold**; RT – retention time; n.d. - not detected

Compound	Identification ions/RT	Royal Glory 2009	Royal Glory 2010	Redhaven 2009	Redhaven 2010	Maria Marta 2009	Maria Marta 2010	Norman 2009	Norman 2010
Alcohols									
3-Hexen-1-ol	41, 67, 55, 39, 82 / 12.99	0.003 (0.002 – 0.005)	0.003 (0.003 – 0.003)	n.d.	0.006 (0.004 – 0.009)	0.003 (0.002 – 0.004)	0.003 (0.002 – 0.004)	n.d.	0.006 (0.004 – 0.007)
*1-Hexanol	56, 43, 41, 55, 42 / 13.18	0.611 (0.393 – 1.083)	0.361 (0.298 – 0.405)	0.387 (0.274 – 0.467)	0.635 (0.542 – 0.709)	0.510 (0.429 – 0.583)	0.417 (0.332 – 0.527)	0.417 (0.254 – 0.596)	0.305 (0.294 – 0.313)
*(E)-2-Hexen-1-ol	57, 41, 67, 39, 82 / 13.27	0.331 (0.265 – 0.467)	0.342 (0.300 – 0.370)	0.283 (0.140 – 0.528)	0.431 (0.340 – 0.569)	0.343 (0.269-0.438)	0.330 (0.238 – 0.477)	0.262 (0.122 – 0.353)	0.211 (0.193 – 0.226)
1-Nonanol	41, 55, 43, 56, 70 / 20.31	n.d.	0.003 (0.003 – 0.003)	n.d.	0.005 (0.005 – 0.005)	n.d.	0.003 (0.003 – 0.003)	n.d.	n.d.
Aldehydes									
*Hexanal	44, 56, 41, 43, 57 / 12.00	3.924 (2.840 – 5.209)	2.296 (2.181 – 2.406)	4.754 (3.400 – 6.068)	4.497 (3.978 – 4.800)	5.137 (4.953 – 5.364)	3.177 (2.605 – 4.266)	4.245 (2.341 – 5.104)	2.551 (2.544 – 2.560)
*(E)-2-Hexenal	41, 55, 69, 39, 83 / 13.42	4.690 (2.655 – 5.688)	3.686 (3.660 – 3.711)	4.761 (2.720 – 5.989)	4.207 (3.716 – 4.642)	5.669 (4.878 – 5.992)	3.515 (2.577 – 4.942)	5.355 (2.310 – 6.991)	3.168 (2.982 – 3.470)
Heptanal	44, 43, 41, 70, 29/ 14.21	0.008 (0.007 – 0.008)	0.004 (0.003 – 0.005)	0.008 (0.008 – 0.010)	0.010 (0.009 – 0.011)	0.009 (0.007 – 0.010)	0.007 (0.005 – 0.008)	0.012 (0.011 – 0.014)	0.005 (0.004 – 0.005)
*(E,E)-2,4-Hexadienal	81, 39, 41, 53, 67 / 15.14	0.409 (0.241 – 0.491)	0.432 (0.424 – 0.438)	0.415 (0.202 – 0.523)	0.546 (0.461 – 0.597)	0.494 (0.412 – 0.561)	0.432 (0.320 – 0.605)	0.486 (0.191 – 0.656)	0.402 (0.366 – 0.474)
(Z)-2-Heptenal	41, 27, 55, 83, 57 / 15.94	n.d.	n.d.	n.d.	n.d.	n.d.	0.007 (0.006 – 0.008)	0.023 (0.003 – 0.034)	0.012 (0.010 – 0.014)
(E)-2-Nonenal	41, 27, 43, 29, 55 / 15.28	0.009 (0.009 – 0.009)	0.014 (0.013 – 0.015)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
*Benzaldehyde	106, 77, 105, 51, 50 / 17.18	0.094 (0.063 – 0.168)	0.114 (0.099 – 0.137)	0.355 (0.294 – 0.405)	0.407 (0.350 – 0.496)	0.252 (0.201 – 0.285)	0.159 (0.126 – 0.192)	0.444 (0.324 – 0.601)	0.240 (0.209 – 0.303)
(E)-2-Octenal	41, 29, 55, 70, 27 / 18.38	n.d.	n.d.	n.d.	n.d.	0.005 (0.005-0.005)	0.006 (0.006-0.006)	0.008 (0.007-0.009)	0.004 (0.003-0.004)
Nonanal	57, 41, 43, 56, 44 / 19.10	0.021 (0.013 – 0.029)	0.017 (0.015 – 0.019)	0.027 (0.022 – 0.031)	0.030 (0.029 – 0.032)	0.022 (0.019 – 0.025)	0.019 (0.016 – 0.024)	0.028 (0.017 – 0.038)	0.022 (0.018 – 0.025)
2,5-Furandicarboxaldehyde	124, 123, 95, 67, 125 / 20.4	0.006 (0.005 – 0.008)	n.d.	0.058 (0.058 – 0.058)	n.d.	0.011 (0.002 – 0.020)	n.d.	0.007 (0.003 – 0.014)	0.004 (0.002 – 0.005)
*Decanal	41, 43, 57, 29, 55 / 21.20	0.002 (0.002 – 0.003)	0.005 (0.004 – 0.005)	0.004 (0.004 – 0.005)	0.008 (0.006 – 0.008)	0.003 (0.003 – 0.004)	0.003 (0.002 – 0.005)	0.005 (0.002 – 0.007)	0.002 (0.002 – 0.003)

Table 1. Continued

Compound	Identification ions/ <i>RT</i>	Royal Glory 2009	Royal Glory 2010	Redhaven 2009	Redhaven 2010	Maria Marta 2009	Maria Marta 2010	Norman 2009	Norman 2010
Ketones									
3-Methyl-2-cyclopenten-1-one	96, 67, 53, 39, 81 / 15.55	0.005 (0.005 – 0.005)	0.009 (0.007 – 0.010)	n.d.	n.d.	0.005 (0.005 – 0.005)	0.009 (0.008 – 0.009)	n.d.	0.007 (0.006 – 0.009)
2,3-Octanedione	43, 99, 71, 41, 55 / 16.13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.007 (0.004–0.009)	0.004 (0.003–0.005)
3(2 <i>H</i>)-Benzo-furanone	134, 105, 76, 77 / 21.69	0.002 (0.001 – 0.003)	n.d.	n.d.	0.003 (0.002 – 0.004)	n.d.	n.d.	n.d.	n.d.
Esters									
Ethyl acetate	43, 29, 45, 61, 27 / 7.86	n.d.	n.d.	0.144 (0.144 – 0.144)	0.029 (0.029 – 0.029)	0.137 (0.137 – 0.137)	n.d.	0.491 (0.491 – 0.491)	n.d.
Pentyl acetate	43, 70, 42, 55, 61 / 14.17	0.018 (0.018 – 0.018)	n.d.	0.017 (0.006 – 0.028)	0.005 (0.005 – 0.005)	0.010 (0.010 – 0.010)	n.d.	0.011 (0.011 – 0.011)	n.d.
*Hexyl acetate	43, 56, 61, 55, 41 / 16.60	1.425 (1.146 – 2.153)	1.230 (0.833 – 1.635)	1.695 (0.985 – 3.405)	1.919 (0.750 – 3.622)	1.615 (0.929 – 2.638)	0.993 (0.841 – 1.269)	1.266 (0.607 – 1.766)	0.930 (0.837 – 1.059)
*3-Hexenyl acetate	43, 67, 82, 41, 39 / 16.69	1.160 (0.918 – 1.497)	1.018 (0.697 – 1.189)	0.546 (0.304 – 0.950)	0.543 (0.474 – 0.657)	0.769 (0.613 – 1.093)	0.712 (0.527 – 0.928)	1.108 (0.405 – 1.587)	0.889 (0.867 – 0.901)
*2-Hexenyl acetate	43, 67, 41, 82, 27 / 16.88	0.764 (0.678 – 0.874)	0.859 (0.693 – 1.056)	1.213 (0.494 – 2.745)	1.470 (0.491 – 2.765)	1.051 (0.477 – 1.737)	0.746 (0.521 – 1.158)	0.891 (0.207 – 1.488)	0.677 (0.626 – 0.729)
Methyl octanoate	74, 87, 43, 41, 55 / 19.29	0.012 (0.007 – 0.023)	0.009 (0.009 – 0.009)	0.017 (0.009 – 0.030)	0.021 (0.021 – 0.021)	0.014 (0.014 – 0.014)	n.d.	0.014 (0.014 – 0.014)	n.d.
*5-Ethyl-2(5<i>H</i>)-furanone	83, 55, 27, 29, 17 / 19.37	0.012 (0.010 – 0.014)	0.009 (0.009 – 0.010)	0.021 (0.020 – 0.022)	0.017 (0.016 – 0.019)	0.016 (0.014 – 0.019)	0.010 (0.005 – 0.018)	0.015 (0.005 – 0.022)	0.006 (0.005 – 0.007)
*γ-Caprolactone	85, 29, 42, 27, 56 / 19.76	0.008 (0.004 – 0.015)	0.002 (0.002 – 0.003)	0.016 (0.011 – 0.019)	0.014 (0.011 – 0.021)	0.014 (0.008 – 0.022)	0.006 (0.005 – 0.008)	0.012 (0.007 – 0.015)	0.007 (0.006 – 0.009)
2-Methylhexyl propanoate	43, 71, 89, 56, 41 / 20.61	0.002 (0.002 – 0.002)	0.004 (0.003 – 0.004)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ethyl octanoate	88, 29, 57, 41, 60 / 20.70	0.002 (0.002 – 0.003)	0.003 (0.002 – 0.003)	0.005 (0.001 – 0.017)	0.010 (0.010 – 0.010)	0.007 (0.007 – 0.007)	n.d.	0.010 (0.010 – 0.010)	n.d.
*2-Hexenyl butanoate	71, 67, 43, 41, 27 / 20.85	0.003 (0.002 – 0.005)	0.013 (0.012 – 0.014)	0.047 (0.001 – 0.179)	0.008 (0.003 – 0.017)	0.004 (0.003 – 0.005)	0.004 (0.002 – 0.007)	0.007 (0.003 – 0.010)	0.004 (0.002 – 0.007)
Octyl acetate	43, 56, 70, 55, 84 / 20.99	n.d.	0.047 (0.032 – 0.062)	n.d.	0.020 (0.005 – 0.044)	n.d.	n.d.	n.d.	n.d.
(<i>E</i>)-2-Hexenyl hexanoate	99, 43, 41, 55, 71 / 24.32	n.d.	0.006 (0.004 – 0.010)	0.005 (0.004 – 0.006)	n.d.	n.d.	n.d.	n.d.	n.d.

Table 1. Continued

Compound	Identification ions/RT	Royal Glory 2009	Royal Glory 2010	Redhaven 2009	Redhaven 2010	Maria Marta 2009	Maria Marta 2010	Norman 2009	Norman 2010
Esters									
γ -Octalactone	85, 29, 57, 56, 41 / 23.67	n.d.	n.d.	n.d.	n.d.	0.002 (0.002–0.002)	n.d.	0.002 (0.002–0.002)	n.d.
* γ -Decalactone	85, 29, 128, 43, 41 / 27.03	0.007 (0.002 – 0.017)	0.002 (0.002 – 0.003)	0.019 (0.002 – 0.050)	0.007 (0.003 – 0.015)	0.010 (0.002 – 0.027)	0.006 (0.004 – 0.008)	0.010 (0.002 – 0.031)	0.003 (0.001 – 0.004)
δ -Octalactone	99, 42, 71, 70, 55 / 27.83	0.004 (0.004 – 0.004)	n.d.	0.010 (0.005 – 0.016)	n.d.	n.d.	–	n.d.	n.d.
Terpenic compounds									
α -Pinene	93, 41, 69, 39, 77 / 15.91	0.007 (0.006 – 0.008)	n.d.	0.020 (0.011 – 0.037)	n.d.	n.d.	n.d.	0.003 (0.003 – 0.003)	n.d.
<i>cis</i> -Geraniol	69, 93, 41, 68, 67 / 15.92	n.d.	n.d.	0.013 (0.010–0.017)	n.d.	n.d.	n.d.	n.d.	n.d.
Limonene	68, 67, 93, 39, 41 / 17.33	0.032 (0.021 – 0.049)	n.d.	0.080 (0.030 – 0.134)	0.017 (0.017 – 0.017)	0.063 (0.021 – 0.105)	n.d.	0.032 (0.020 – 0.044)	0.010 (0.010 – 0.010)
α -Cymene	119, 134, 91, 120 / 17.54	0.010 (0.007 – 0.013)	n.d.	0.028 (0.018 – 0.045)	0.014 (0.014 – 0.014)	0.009 (0.009 – 0.009)	n.d.	0.014 (0.008 – 0.018)	n.d.
*Linalool	71, 93, 41, 69, 43 / 18.91	0.125 (0.046 – 0.205)	0.029 (0.023 – 0.038)	0.389 (0.250 – 0.493)	0.064 (0.043 – 0.095)	0.048 (0.031 – 0.081)	0.007 (0.003 – 0.013)	0.104 (0.030 – 0.211)	0.020 (0.009 – 0.030)
α -Terpineol	59, 93, 121, 136 / 21.62	0.002 (0.002 – 0.002)	n.d.	0.003 (0.002 – 0.003)	n.d.	n.d.	n.d.	0.003 (0.003 – 0.003)	n.d.
Other									
Hexanoic acid	60, 73, 41, 27, 43 / 15.4	0.008 (0.008 – 0.008)	0.014 (0.013 – 0.015)	0.019 (0.008 – 0.030)	0.029 (0.025 – 0.032)	0.012 (0.008 – 0.019)	0.016 (0.009 – 0.023)	0.010 (0.003 – 0.014)	0.011 (0.010 – 0.012)
2-Ethylfuran	81, 53, 96, 39, 41 / 9.64	0.021 (0.014 – 0.031)	0.058 (0.055 – 0.062)	0.025 (0.016 – 0.029)	0.060 (0.054 – 0.069)	0.030 (0.024 – 0.035)	0.047 (0.024 – 0.074)	0.039 (0.016 – 0.079)	0.053 (0.040 – 0.072)
*2-Pentylfuran	81, 82, 27, 53, 95 / 16.25	0.010 (0.006 – 0.015)	0.011 (0.010 – 0.011)	0.014 (0.012 – 0.020)	0.016 (0.014 – 0.018)	0.013 (0.009 – 0.016)	0.007 (0.005 – 0.010)	0.017 (0.011 – 0.021)	0.010 (0.008 – 0.012)
5-(Hydroxymethyl)-2-furan-carboxaldehyde	97, 126, 41, 39, 69 / 23.3	n.d.	n.d.	0.120 (0.001 – 0.238)	n.d.	0.028 (0.028 – 0.028)	n.d.	0.006 (0.001 – 0.011)	n.d.

man”, our predominant task was to perform the analysis, which would offer us a discrimination of cultivars using the same compounds present in all peach cultivars during two completely different seasons (the amount of rainfall). For this purpose we applied the discriminant analysis using the compositional data marked with stars (*) in Table 1 and the data regarding

the composition of sugars (glucose, fructose, sucrose and sorbitol) and acids (citric and malic) in peaches. By applying the multiple group discriminant analysis, it is not necessary to specify how to combine groups so as to form different discriminant functions. The first function provides the most overall discrimination between groups; the second provides second most,

and so on. Moreover, the functions will be independent or orthogonal, and their contributions to the discrimination between groups will not overlap. The analysis included three discriminant functions, of which all three have significant contribution to group differentiation.

Function 1, which accounts for 56.9% of the variance explained by the model, is more heavily weighted by citric acid, linalool, (*E*)-2-hexenal and benzaldehyde (Table 2). Thus, function 1 separates “Royal Glory” cultivar with its sweet fruits from “Redhaven” cultivar which has much sour and aromatic fruits (Fig. 1). In fact, the most discriminative component in “Royal Glory” peaches is glucose, whereas in “Redhaven” peaches is linalool, with its floral, fresh note and benzaldehyde with almond-like odor. Both classifications of peach cultivars are in complete accordance with sensory evaluations.

Table 2. Standardized canonical discriminant function coefficients for the first three functions

Compound	Function 1	Function 2	Function 3
1-Hexanol	0.369	0.986	0.398
(<i>E</i>)-2-Hexenal	-1.212	-0.872	-0.925
(<i>Z</i>)-3-Hexenyl acetate	-0.560	-1.019	0.362
Benzaldehyde	1.083	0.119	1354
α -Linalool	1.350	1.047	1.244
γ -Caprolactone	0.237	0.392	-1.194
Citric acid	1.706	-1.127	-0.322
Sucrose	0.631	-1.250	0.803
Glucose	-0.797	1.870	0.359
Eigenvalue	18.83	10.48	3.79
% Of explained variance	56.9	31.7	11.5

The second function accounts for another 31.7% of the explained variance and seems to be associated mostly with glucose and to a less extent by sucrose, citric acid, linalool, 1-hexanol, (*E*)-2-hexenal and (*Z*)-3-hexenyl acetate. Hence function 2 separates sensorial similar cultivars “Norman” and “Maria Marta” from “Redhaven” and “Royal Glory” cultivars, but this separation is not as strong as the separation caused by function 1 (Fig. 1). The most discriminative coefficients were for both cultivars lower values of glucose and higher values of sucrose and citric acid.

Function 3 also significantly contributes to group differentiation (data not shown). Its contribution to the explained variance is 11.5% but the relatively low value of eigenvalue shows weak discriminant ability, so its interpretation is less important.

The impact of preservatives (citric acid vs. sodium benzoate) on the aromatic profile of cooked peaches

Cooking by itself has an important impact on the aromatic profile of the peaches and it has been studied in details also by other authors [12,13,17]. Cooking of peaches at 95 °C for 30 min is one of many procedures which could be applied, but it is maybe one of the most applicable for household scale, and that was the reason why we have chosen it. In the first place, we were interested in the promoting or/and the masking effect of preservatives on the overall aroma of peaches used as canned peaches, jams or other products. In our study we used two common preservatives citric acid and sodium benzoate.

The statistical analysis indicated that from 57 identified volatiles (Table 3), 40 volatiles showed statistically significant difference regarding the way of preser-

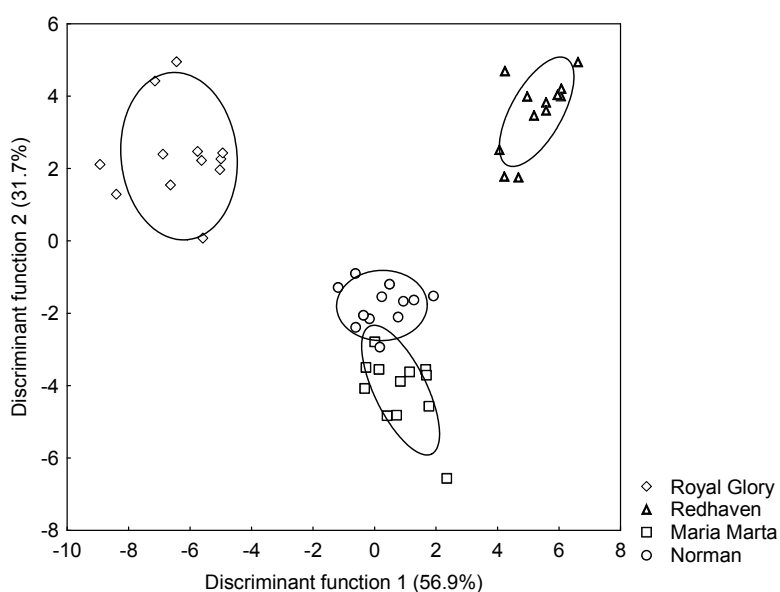


Figure 1. Distribution of the first two discriminant functions according to the stepwise discriminant analysis of peach aromatic profile. The ellipses delimit the space that represents a particular peach cultivar ($p = 0.95$).

Table 3. Arithmetic means of identified volatiles expressed as concentration of internal standard 1-octanol (mg/kg); means followed by the same letter in the same row are not significantly different at $p > 0.05$ according to Tukey's test; * $0.01 < p < 0.05$; ** $0.001 < p < 0.01$; *** $p < 0.001$

Compound	Peach variety	Control		Citric acid		Na benzoate		Interaction
Alcohols								
4-Methyl-cyclohexanol	***	0.0057	A	0.0053	A	0.0032	B ^b	*
3-Hexen-1-ol	**	0.0030	B	0.0023	A	0.0024	AB	***
1-Hexanol		0.1888	A	0.1796	A	0.1374	B	
(E)-2-Hexen-1-ol	*	0.1843	B	0.1654	AB	0.1365	A	
1-Nonanol	***	0.0011		0.0010		0.0011		***
Aldehydes								
(E)-2-Pentenal	***	0.0020	A	0.0020	A	0.0016	B	
Hexanal		1.1799	AB	1.3580	B	1.0874	A	
(E)-2-Hexenal	*	1.6761		1.7045		1.5955		
Heptanal	***	0.0179	B	0.0166	AB	0.0136	A	
(E,E)-2,4-Hexadienal		0.2078	A	0.1973	A	0.1653	B	
(Z)-2-Heptenal	**	0.0326	A	0.0295	A	0.0177	B	
(E,E)-2,4-Heptadienal	***	0.0020		0.0018		0.0017		*
Benzaldehyde	***	0.1569	A	0.1490	A	0.1776	B	
Octanal	***	0.0493	A	0.0496	A	0.0345	B	***
(E)-2-Octenal		0.0214		0.0203		0.0190		
Benzeneacetaldehyde	***	0.0042		0.0038		0.0036		
Nonanal	***	0.4163	B	0.3592	A	0.3199	A	
(E)-2-Nonenal		0.0103		0.0102		0.0092		
Decanal		0.0342	A	0.0305	A	0.1236	B	
2,5-Dimethyl-benzaldehyde	***	0.0030	A	0.0029	A	0.0127	B	***
<i>p</i> -Menth-1-en-9-al		0.0410	A	0.0641	B	0.0410	A	*
(E)-2-Decenal		0.0080	A	0.0088	A	0.0123	B	
Undecanal	***	0.0059		0.0048		0.0067		
3-Ethylbenzaldehyde	***	0.0011	A	0.0011	A	0.0036	B	***
β -Dodecanal	***	0.0009	A	0.0012	B	0.0011	B	***
Ketones								
2,3-Octanedione	**	0.0071	A	0.0073	A	0.0059	B	
3-Methyl-cyclopenten-1-one	**	0.0007		0.0003		0.0000		*
Esters								
Hexyl acetate		0.7860	B	0.5974	A	0.4408	A	
3-Hexenyl acetate	***	0.6332	A	0.5362	A	0.3932	B	
2-Hexenyl acetate	*	0.6191	B	0.5167	AB	0.3943	A	
Ethyl octanoate	***	0.0025	A	0.0032	B	0.0021	A	*
5-Ethyl-dihydro-2(3H)-furanone	**	0.0075	A	0.0075	A	0.0059	B	**
5-Ethyl-2(5H)-furanone	***	0.0171		0.0169		0.0132		
2-Methylhexyl propanoate	***	0.0010		0.0010		0.0008		
Esters								
(Z)-2-Hexenyl butanoate	***	0.0064		0.0053		0.0045		

Table 3. Continued

Compound	Peach variety	Control	Citric acid		Na benzoate		Interaction	
Esters								
(<i>E</i>)-2-Hexenyl hexanoate	***	0.0004		0.0005		0.0009		
Octyl acetate	***	0.0095		0.0095		0.0078		
γ -Decalactone	***	0.0041	A	0.0058	B	0.0046	A	***
Terpenic compounds								
α -Terpinyl acetate	***	0.0187	A	0.0183	A	0.0133	B	*
α -Cymene	***	0.0160	A	0.0160	A	0.0099	B	**
α -Linalool	***	0.0418		0.0448		0.0407		
2,6-Dimethyl-3,7-octadiene-2,6-diol	***	0.0057	AB	0.0078	B	0.0049	A	
α -Terpineol	**	0.0037	A	0.0042	A	0.0154	B	
α -Cyclocitral	***	0.0038	A	0.0046	A	0.0080	B	
α -Ionene	**	0.0011		0.0014		0.0011		
β -Damascenone	***	0.0053	A	0.0099	B	0.0041	A	***
Others								
Tetrahydrofuran	*	0.0037	B	0.0031	AB	0.0015	A	*
Hexanoic acid		0.0070	A	0.0070	A	0.0042	B	
2-Ethylfuran	*	0.0148		0.0134		0.0142		
2-Pentylfuran	*	0.1058	B	0.0797	A	0.0686	A	
3,6-Dihydro-4-methyl-2-(2-methyl-1-propenyl)-2H-pyran	***	0.0088	A	0.0103	A	0.0063	B	
Benzene	*	0.0028	A	0.0038	A	0.0423	B	*
Benzoic acid		0.0000	A	0.0000	A	0.9853	B	
1,2,3,4-Tetrahydro-1,6,8-trimethyl-naphthalene	***	0.0007		0.0007		0.0004		
1,2-Dihydro-1,1,6-trimethyl-naphthalene	***	0.0059	A	0.0092	B	0.0046	A	
Biphenyl	***	0.0000	A	0.0000	A	0.0056	B	***
2-Methyl-1,1'-biphenyl	***	0.0000	A	0.0000	A	0.0158	B	***

vation. The interaction was expressed in 20 cases, but it arises because the particular volatile was found only in one or two peach varieties, whereas in other it was absent. In other words, the interaction occurs when the effect of preservation (on the quantity, presence or absence of volatiles) is changed according to the peach varieties.

In case of 25 volatiles the treatment with sodium benzoate resulted in a significantly different amount of volatiles compared to control samples and samples treated with citric acid. In fact, 11 were of higher values and 14 were of lower. The preservation with citric acid had significantly different contribution in case of 5 volatiles, compared to the control or samples treated with sodium-benzoate. The increase was noticed in the

case of: hexanal, *p*-menth-1-en-9-al, ethyl octanoate, γ -decalactone, and the terpenic compounds 2,6-dimethyl-3,7-octadiene-2,6-diol and β -damascenone. Hexanal, ethyl octanoate and γ -decalactone are compounds typical for peach aroma, already discussed, whereas β -damascenone (cooked-apple-like) and *p*-menth-1-en-9-al were not found in fresh peaches, but they are formed during thermal processing [17,41]. The 2,6-dimethyl-3,7-octadiene-2,6-diol was found in larger amount in Darjeeling tea [42], but it is the first report about its presence in cooked peaches.

Sodium benzoate had the maximal impact on the aldehydes group (on 10 compounds from 20 identified). In fact, its addition decreased the concentration of several aldehydes, like: (*E*)-2-pentenal, heptanal,

(*E,E*)-2,4-hexadienal and (*Z*)-2-heptenal and octanal, while in case of benzaldehyde, decanal, 2,5-dimethylbenzaldehyde, (*E*)-2-decenal and 3-ethylbenzaldehyde it had a positive effect, because the concentration increased. The presence of sodium-benzoate had a negative effect on two esters: (*Z*)-3-hexen-1-ol and 5-ethylidihydro-2(3*H*)-furanone. In the group of terpenic compounds, two of them (α -terpinyl acetate and α -cymene) had lower concentrations than control and samples preserved with citric acid, and two had significantly higher concentrations (α -terpineol and α -cyclocitral). Further, in Table 3, we can notice the increase in concentration of noxious compounds, like: benzene, benzoic acid (known to provoke urinary, angioedema, asthma and childhood hyperactivity [43]), 2-methyl-1,1'-biphenyl and biphenyl genotoxic to mice [44]. This may cause a less pronounced typical peach odor, and undesirable components.

7 volatile compounds from 57 identified were significantly higher in control samples. In this group can be classified two alcohols: 3-hexen-1-ol (grassy green), (*E*)-2-hexen-1-ol (green banana), two aldehydes: heptanal (fresh, green and citrus) and nonanal (fruity and floral), two esters: hexyl acetate (fruity and floral), 2-hexenyl acetate, as well as 2-pentylfuran (green and fresh). All compounds were already identified in fresh peaches, each having an individual aromatic impact, mostly characterized by fresh notes.

CONCLUSIONS

Differences in peach volatiles regarding seasons and variety were observed in all 4 cultivars. Groups of volatiles were specifically susceptible to monthly rainfall; however a general increase was noticed in the case of some aldehydes, esters, all identified lactones and terpenes in season with less rainfall. Beside seasonal also cultivars differences were observed and statistically evaluated. By applying the multiple group discriminant analysis, it was possible to discriminate all 4 cultivars by two functions. Function 1, which accounted for 56.9% of the variance explained by the model, was influenced more heavily by citric acid, linalool, (*E*)-2-hexenal and benzaldehyde and it was able to separate "Royal Glory" from "Redhaven". The second function, which accounted for another 31.7% of the explained variance, seems to be mostly associated with glucose content and to a less extent by sucrose, citric acid, linalool, 1-hexanol, (*E*)-2-hexenal and (*Z*)-3-hexenyl acetate was able to separate similar cultivars "Norman" and "Maria Marta".

Cooking by itself, in the other hand has an important impact on the aromatic profile of peaches. However, our interest was oriented in those, which were promoted or/and masked by the added preservatives. The statistical analysis indicated that from 57 identified

volatiles, 40 volatiles showed statistically significant difference regarding the way of preservation. In case of 25 volatiles the treatment with sodium benzoate resulted in a significantly different amount of volatiles compared to control samples and samples treated with citric acid. Sodium benzoate had the maximal impact on the aldehyde group (on 10 compounds from 20 identified), a negative effect on two esters: (*Z*)-3-hexen-1-ol and 5-ethylidihydro-2(3*H*)-furanone. Terpenic compounds reacted in the presence of sodium-benzoate differently, two of them (α -terpinyl acetate and α -cymene) were of lower concentration, and two were of significantly higher concentration (α -terpineol and α -cyclocitral) than those observed in the case of control samples and samples preserved with citric acid. The most important and relevant observation came from the fact that the addition of sodium-benzoate caused the increase in concentration of noxious compounds, like: benzene, benzoic acid, 2-methyl-1,1'-biphenyl and biphenyl. All compounds are known to cause adverse health effects. The preservation with citric acid had significantly different contribution in case of only 4 volatiles, and 7 volatile compounds were significantly higher in control samples.

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IZVOD

AROMATIČNE MATERIJE RAZLIČITIH SORTI BRESAKA I UTICAJ KONZERVANASA NA FINALNU AROMU KUVANOG VOĆA

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Breskva je sočan plod drveta *Prunus persica*. Aromu bresaka čine više stotina hemijskih jedinjenja koja pripadaju različitim klasama (alkoholi, estri, aldehidi, ketoni, laktoni, terpena jedinjenja, itd.). Među materijama koje definišu aromu, kao predstavnici "zelene, nesazrele arome" se ističu (*E*)-2-heksenol i (*E*)-2-heksenal, dok su glavna jedinjenja koja definišu voćnu, zrelu aromu γ -dekalakton i γ -dodekalakton. Tokom sazrevanja sadržaj C6 jedinjenja (heksanala, (*E*)-2-heksanala) opada, dok koncentracija laktone raste. Sazrevanje bresaka i razvoj tipične arome bresaka jako zavisi od sadržaja laktone, (δ - i γ -dekalaktona). U ovom radu, tokom dvogodišnjeg perioda ispitane su aromatične materije sorti bresaka mesa žute boje, Royal Glory, Redhaven, Maria Marta i Norman. Metoda mikroekstrakcije u čvrstoj fazi – uzorkovanje iz gasovite faze (HS-SPME) je uspešno primenjena za određivanje isparljivih jedinjenja. Cilj našeg rada je bio da se na osnovu rezultata analiza tokom dvogodišnjeg perioda definišu jasne razlike između ispitivanih sorti u pogledu identifikovanih jedinjenja. Takođe, ispitivan je uticaj konzervanasa, natrijum-benzoata i olimunske kiseline, na aromu kuvanih bresaka. Sadržaj isparljivih komponenata je zavisio od mesečnih padavina, odnosno došlo je do povećanja sadržaja nekih aldehida, estara, kao i laktone i terpena u sezoni sa manje padavina. Međutim, pored razlike u sezonama, utvrđene su i razlike između ispitivanih sorti. U cilju razdvajanja – diskriminacije ispitivanih sorti na osnovu aromatičnog profila, korišćena je statistička diskriminantna analiza višestrukih grupa. Iako su jako slične, sorte su razdvojene na osnovu 2 funkcije, funkcije 1 (koja čini 56,9% po primenjenom modelu) i funkcije 2 (31,7%). Sa druge strane, upotreba konzervanasa ispoljava statistički značajan uticaj na aromatski profil kuvanih bresaka. Naime, od 57 identifikovanih isparljivih jedinjenja, kod 40 je utvrđena statistički značajna razlika u pogledu načina konzerviranja. Utvrđeno je da je glavni negativan uticaj kao konzervans ispoljio natrijum-benzoat, u poređenju sa kontrolnim uzorkom i uzorcima kod kojih je dodata limunska kiselina.

Ključne reči: Aroma bresaka • Isparljive komponente bresaka • Diskriminantna analiza • Prehrambeni konzervansi • Mikroekstrakcija u čvrstoj fazi