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Identification of Biphenyls – Contaminants Responsible for Off-Flavour in Soft Drinks

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Abstract

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Off-flavour in soft drinks is one of the main threats to manufacturers, which can result in expensive recalls, and discredit the brand. Off-flavours can occur for a variety of reasons (e.g. chemical contamination of raw material). The presented case study has proved there is a relationship between the identification of biphenyl and its derivatives in the used preservative (benzoate) and off-flavours in the drink. The project consisted of three phases: (1) the assessment of the probable cause of off-flavours based on sensory evaluation and GC-MS-Olfactometry profiling of volatiles; (2) the quantification of biphenyls and the characterisation of their sensory properties; (3) the screening of commercially available benzoates for the presence of biphenyls. Based on the odour threshold obtained by GC-O (0.03 mg/l for 4-methyl-1,1'-biphenyl and 0.02 mg/l for biphenyl in an aqueous solution) and the common benzoate content in soft drinks (0.14 g/l), the 'non-observable sensory' levels of contamination were determined to be maximally 0.143 mg/g of benzoate for biphenyl and 0.214 mg/g of benzoate for 4-methyl-1,1'-biphenyl .

Keywords: non-alcoholic beverages; olfactometry; preservatives; sensory defects; sodium benzoate; taint

Soft drinks are generally considered to be stable and resistant to unwanted changes and spoilage. However, from time to time sensory defects can occur in soft drinks, whether in the form of altered smell, taste and/or appearance, which leads to consumer complaints. According to some authors (Ridgway *et al.* 2009), the defects originating from the environment and caused exclusively by contamination are called taints, whereas the defects formed in the food or beverage due to degradation of its components are called off-flavours. However, this distinction is seldom made, particularly in consumer complaints (Ridgway *et al.* 2009). Sensory defects in soft drinks can be caused by: (a) chemical and enzymatic changes of ingredients; (b) microbial contamination and subsequent microbial decay; (c) non-compliance with manufacturing processes; (d) chemical contamination; (e) improper storage; and (f) a combination of the above (Widén *et al.* 2005; Rouseff & Naim 2007; Perez-Cacho & Rouseff 2008; Čížková *et al.* 2009; Horsáková *et al.* 2009; Juvonen *et al.* 2011). Soft drink manufacturers try to eliminate the incidence of sensory defects by carefully selecting raw materials, choosing suitable and sufficient preservation methods and types of packaging materials, and adhering carefully to management control systems (HACCP, GMP, and GHP).

Commonly detected chemical contaminants in soft drinks coming from packaging materials include styrene and acetaldehyde (Ridgway *et al.* 2009); many off-flavours are caused by detergents and disinfectants (Ridgway *et al.* 2009; Ma *et al.* 2016); and some defects can also be caused by contamination from the manufacturing plant or a maintenance action (Čížková *et al.* 2009).

Benzoic acid and its salts are among commonly used preservatives in the beverage industry. Sodium

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benzoate (E211, SB) is an odourless white crystalline powder with various tastes depending on its concentration and human perception (Amerine *et al.* 2013). An SB aqueous solution in concentrations up to 0.1% has nonlinear taste intensity of the four basic tastes (salt and sweet tastes increase, bitterness first decreases, and then increases, sour changes a little); afterwards the overall taste intensity is linear. Benzoic acid, a precursor of benzoate, is exclusively (WHO 2000) made by liquid-phase oxidation of toluene by air (Hundley & Nathan 1965). This can also create numerous by-products, including biphenyl and its derivatives. The purity of the final benzoate depends on the conditions and subsequent purification. No information about either biphenyl or some of its derivatives causing off-flavours in foods and beverages is available in literature.

The first step in the detection and identification of off-flavours in samples or in determining if their flavour (qualitatively and quantitatively) matches a control sample is a sensory analysis. Besides the classic sensory analysis, modern analytical methods using different types of sensors are recommended, i.e. an electronic nose (Bleibaum *et al.* 2002; Wei *et al.* 2017), or a combination of gas chromatography and olfactometry (GC-O) (Högnadóttir & Rouseff 2003; Plutowska & Wardencki 2008). GC-O was used in the presented case study to identify off-flavour origin in recalled soft drinks.

MATERIAL AND METHODS

Chemicals and samples. A sample of an off-flavour soft drink with strawberry flavour (composition: natural mineral water, sugar, flavouring, citric acid, SB, ascorbic acid), which was subjected to consumers' complaints, was obtained from a Czech manufacturer together with a control sample; with the same strawberry flavour and composition, but without any sensory defects, and eight samples of SB (synthetic origin, declared purity min. 99%, production year 2015) (Donauchem and Brenntag CR, Czech Republic). The standards of SB (> 99%), 4-phenyltoluene (syn. 4-methyl-1,1'-biphenyl, 98%), biphenyl (≥ 99%), and *n*-alkanes (C8-C20, used for retention index calculations) were purchased from Sigma-Aldrich (Czech Republic). Ethanol (p.a., 96%) was purchased from Penta (Czech Republic).

Sensory evaluation. Sensory evaluation was performed by the total of 10 assessors from UCT (Czech Republic), each assessor evaluating three sets of tests. The 'A'–'not-A' test (for odour and taste, according to ISO 8588, 1987) was used to distinguish between the off-flavour sample and the control sample of the soft drink. The triangle test (for odour and taste, according to ISO 4120, 2004) was used to distinguish between the suspicious SB samples provided by the manufacturer and the SB standard.

The forced-choice triangle test was used to determine the odour threshold of biphenyls, testing aqueous solutions with increasing concentrations (0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.1, and 0.2 mg/l) (Leitnerová *et al.* 2010). The minimum concentration at which 50% of the assessors could detect the presence of an odour was established as the odour threshold (Brattoli *et al.* 2013).

SPME-GC-MS analysis. The analysis was performed according to Šístková *et al*. (2017). The isolation of the substances was performed by Solid Phase Microextraction (SPME) on the DVB/Carboxen™/PDMS StableFlex™ fibre (50/30 μm; Supelco, USA). Five ml aliquot of each of the samples was transferred into a 10 ml vial. The samples were tempered by being stirred for 1 min at 40°C, extraction time was 30 min at 40°C, desorption time was 4 min at 240°C, and the split ratio was 1 : 1. The separation was performed on the 7890B gas chromatograph with the 5977A mass detector and the HP-5MS 5% phenyl methyl siloxane column (30 × 250 × 0.25 μm), all from Agilent Technologies (USA). Helium was used as the mobile phase at the constant flow rate of 1.4 ml/minute. The temperature programme was as follows: 60°C for 2 min, then 10°C/min up to 320°C, and held for 2 minutes. The total time of analysis was 30 minutes. The MS detector temperatures were set as follows: the source temperature –230°C, the quadrupole temperature –150°C, the transfer line temperature –280°C.

Volatiles were identified by comparing the mass spectra with the NIST 08 MS spectra database (National Institute of Standards and Technology, USA), and the calculated retention indices (RI) (Van den Dool & Kratz 1963). A calibration curve was used for the quantification of the available standards (biphenyl and 4-methyl-1,1'-biphenyl). Abundance (%) and peak area/1000 were used to show the differences between the amounts of other derivatives in the samples. All the samples were analysed three times, the average results are presented. The profiles of the volatile compounds were evaluated based on their relative representation; repeatability, expressed as relative standard deviation (RSD), was less than 8%.

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GC-O analysis. The sensory properties of the isolated volatile substances were simultaneously evaluated by an olfactometry analysis. The GC effluent was split 1 : 1 between the MS (as described above) and an olfactometer (JAS, Moers, Germany). The olfactory port was heated to 180°C; the sniffing port was supplied with humidified air with a constant flow (30 ml/min). A binary signal (present/absent) was recorded by pushing a button; at the same time an odour description was recorded by a panel of 6 trained assessors (UCT, Czech Republic), who had previous experience with GC-O.

A detection frequency method, the nasal impact frequency (NIF, each of the *n* assessors contributes 1/*n* to the final result), was used for the soft drink samples, the SB samples and the standards. Substances detected only once were deemed noise; substances with the detection frequency of ≥ 2 were considered to be sensory active (Pang *et al.* 2012).

The odour activity value (OAV) was determined by a dilution analysis method. A solution containing 1 mg/l of the biphenyl standard and 1 mg/l of the 4-methyl-1,1'-biphenyl standard was gradually diluted until the odour thresholds of both substances were reached (Pang *et al.* 2012).

HPLC analysis. The purity of the SB samples was determined by HPLC/DAD according to Grégrová *et al.* (2014).

RESULTS AND DISCUSSION

Preliminary sensory evaluation. Compared to the control sample, the off-flavour sample of the strawberry flavoured soft drink supplied by the manufacturer showed sensory defects, namely altered smell and taste. This difference was confirmed in the laboratory by using the 'A'–'not-A' test ($P < 0.1$). The odour of the off-flavour sample was not perceptible, but its taste was unpleasant and pungent, with chemical aftertaste on the back of the tongue and the palate. The data obtained from the manufacturer's traceability system suggested that the sensory defect was caused by the used preservative – sodium benzoate (its concentration in the soft drink was 0.14 g/l).

The suspicious batch of SB (sample 1) was tested in two concentrations: 0.2 g/l and 0.1 g/l (aqueous solutions). The triangle test confirmed that the difference between the suspicious SB and the SB standard was apparent in the 0.2 g/l concentration ($P < 0.05$). The assessors described the characteristic odour of the suspicious SB as fruity, not pungent, and rather pleasant and its characteristic taste as fruity, resinous or disinfection-like. No significant differences were recorded in the 0.1 g/l concentration ($P > 0.1$).

SPME-GC-MS and GC-O analysis. Using gas chromatography together with olfactometry produced rather similar profiles of volatiles in both

Table 1. Odour active substances of the tested soft drinks (off-flavour, control) and the suspicious batch of sodium benzoate (SB)

Substance No.	RT[a] (min)	Substance	RI[b]	Description of smell[c]	Relative abundance (%)			NIF[d]		
					control	off-flavour	suspicious batch of SB (sample 1)	control	off-flavour	suspicious batch of SB (sample 1)
1	3.94	ethyl butanoate	804	sweet, strawberry	6.9	8.0	nd	0.67	0.67	nd
2	4.71	ethyl 3-methylbutanoate	852	sweet, fruity	13.7	14.3	nd	1.00	1.00	nd
3	7.20	ethyl hexanoate	1005	fruity, raspberry	32.0	35.7	nd	0.67	0.67	nd
4	7.42	hexyl acetate	1018	sweet, raspberry	14.9	17.3	nd	0.50	0.17	nd
5	8.17	unidentified	1065	sweet, sugar	nd	nd	nd	0.50	0.50	nd
6	8.88	isopentyl isovalerate	1111	sweet, chocolate	14.2	13.0	nd	0.67	0.33	nd
7	12.72	3-hexenyl hexanoate	1386	sweet, strawberry	0.1	0.1	nd	0.50	0.33	nd
8	12.83	methyl cinnamate	1394	sweet, fruity	2.2	1.8	nd	0.33	0.50	nd
9	13.01	2-methyl-1,1'-biphenyl	1408	artificial, medical	< 0.1	< 0.1	47.3	nd	0.33	1.00
10	14.19	4-methyl-1,1'-biphenyl	1503	artificial, plastic	< 0.1	< 0.1	10.6	nd	0.5	1.00

[a]retention time; [b]calculated retention indices for a DB5 column; [c]perception description obtained from the panel of assessors (GC-O); [d]nasal impact frequency (NIF – each of the 6 assessors contributes 1/6 to the final result; only substances with NIF ≥ 0.50; i.e. ≥ 3 assessors detected the substance for at least one sample are shown); nd – not detected

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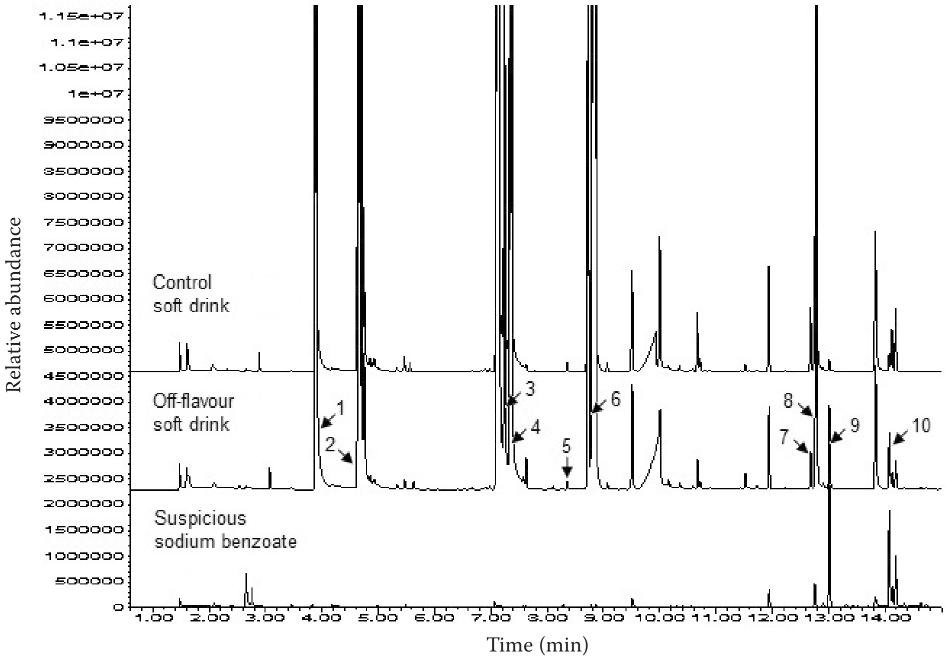

Figure 1. The profile of volatiles of the off-flavour sample and the control sample of the soft drink and the suspicious batch of SB (sample 1). The numbers correspond to the odour active substances summarised in Table 1

the off-flavour and the control sample (Figure 1 and Table 1). The major volatiles in both the samples were ethyl 3-methylbutanoate (RT = 4.71 min), ethyl hexanoate (RT = 7.20 min), and hexyl acetate (RT = 7.42 min). These are all substances having a sweet, fruity flavour. However, the off-flavour sample also contained biphenyl (RT = 12.75 min) and two of its derivatives: 2-methyl-1,1'-biphenyl (RT = 13.01 min), and 4-methyl-1,1'-biphenyl (RT = 14.19 min). While the biphenyl derivatives were detected by GC-O in the off-flavour sample, biphenyl itself was co-eluting with 3-hexenyl hexanoate (RT = 12.72 min), whose sweet strawberry aroma predominated in the GC-O.

The analysis of the suspicious batch of SB subsequently confirmed the occurrence of biphenyl and its derivatives. All of the 6 trained assessors recorded the elution of the derivatives during GC-O (NIF = 1). Using a calibration curve, the biphenyls contained in 1 g of the SB sample were quantified as follows: biphenyl 0.17 mg; 4-methyl-1,1'-biphenyl 0.40 mg. This corresponds to 0.024 mg of biphenyl and 0.056 mg of 4-methyl-1,1'-biphenyl in 1 litre of the soft drink.

Determination of the odour threshold of biphenyls. It is important to determine the odour threshold of biphenyls in order to determine 'non-observable sensory' levels in cases of SB contamination, i.e. the

Table 2. Odour and flavour characteristics, odour threshold, and odour activity value (OAV) for biphenyl and 4-methyl-1,1'-biphenyl

	Odour characteristics[a]	Flavour characteristics[a]	Odour threshold in water (mg/l)			OAV[c]		
			from literature[b]	from sensory evaluation	from GC-O	control soft drink	off-flavour soft drink	suspicious batch of SB (c = 0.1 g/l)
Biphenyl	pungent, rose, green, geranium	neroli, bergamot, cinnamon, natural	0.00052–0.0095	0.02	0.02	< 0.7	1.6	0.8
4-methyl-1,1'-biphenyl	spicy, estragole, fennel, floral, wintergreen	floral, spicy, wintergreen, estragole and waxy with cooling, minty nuances	not found	0.03	0.03	< 0.5	1.9	1.3

[a]TGSC (2017; thegoodscentscompany.com); [b]Murnane (2013); [c]odour activity value (the ratio of the concentration in the sample to the odour threshold from the sensory evaluation; OAV ≥ 1 were considered to be potential contributors to aroma profiles); SB – sodium benzoate

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Table 3. Screening of the available benzoates for the presence of biphenyl and its derivatives

Name of biphenyls	RT (min)	RI	Sample of SB							
			1	2	3	4	5	6	7	8
Biphenyl	12.75	1388	522	106	806	1100	nd	288	nd	nd
2-Methyl-1,1'-biphenyl	13.01	1408	1919	147	196	1297	nd	956	nd	nd
Diphenylmethan	13.43	1442	12	nd	34	6	nd	6	nd	nd
3-Methyl-1,1'-biphenyl	14.08	1494	1824	451	508	3235	47	1033	28	16
4-Methyl-1,1'-biphenyl	14.19	1503	935	291	243	1672	23	535	nd	8
2,4'-Dimethyl-1,1'-biphenyl	14.34	1516	37	9	6	92	nd	19	nd	nd
2,2'-Dimethyl-1,1'-biphenyl	14.73	1549	26	2	14	20	nd	15	nd	nd
Content of biphenyl (mg/g)			0.17	< 0.1	0.26	0.35	< 0.1	< 0.1	< 0.1	< 0.1
Content of 4-methyl-1,1'-biphenyl (mg/g)			0.4	0.12	0.1	0.72	< 0.1	0.23	< 0.1	< 0.1

Sample 1 – sodium benzoate (SB) used in the off-flavour soft drink; samples 2–7 – SB of different origin; sample 8 – the laboratory standard of SB; nd – not detected; the volatiles identified in the analysed SB are presented as peak area/1000, the content of biphenyl and 4-methyl-1,1'-biphenyl was quantified by calibration curves (average results from three measurements are presented); RT – retention time; RI – retention indices

amount which does not affect drinks. The threshold concentrations of biphenyls (the available analytical standards, i.e. biphenyl and 4-methyl-1,1'-biphenyl) in water were determined by the triangle test and GC-O (Table 2). Both the substances were distinctly recognized in the concentration of 0.05 mg/l (90% of the answers were correct). As a result, the following 'non-observable sensory' levels of SB contamination (for a recipe containing 0.14 g/l) were determined: 0.143 mg of biphenyl and 0.214 mg of 4-methyl-1,1'-biphenyl in 1 g of SB. These threshold concentrations are applicable to water; in acidified aromatized drinks detectable concentrations of biphenyls would probably be higher. The odour of the off-flavour drink and the suspicious SB was negatively influenced by the presence of biphenyl derivatives. The odour activity value for 4-methyl-1,1'-biphenyl in the off-flavour drink was 1.9; for SB in an aqueous solution, it was 1.3; odour activity values higher than one were considered to be potential contributors to an aroma profile.

Screening of the available benzoates for the presence of biphenyl and its derivatives. For the above reasons, commercially available batches of SB were tested for the presence of biphenyls and compared to the laboratory standard. As Table 3 shows, the analysed benzoates differed in which biphenyls they contained and their respective amounts. The manufacturer's traceability system showed that off-flavour was caused not only by sample 1, but also by sample 4, which had the highest content of biphenyls. The overall contamination of these SB samples with biphenyls was estimated to be max. 3 mg/g. As a result, the conventional SB purity test carried out by HPLC UV/VIS (AOAC Official Method 994.11, 2000) cannot, unfortunately, detect it. All of the SB samples were screened by HPLC and corresponded to the declared purity of 98–100%.

CONCLUSIONS

Biphenyl and its derivatives are by-products of benzoic acid and subsequently contaminate SB, which is used in the beverage industry (Grégrová *et al.* 2014). Although the detected biphenyls in the soft drinks do not probably pose a health risk for consumers, because their concentrations are far from reaching the oral reference dose for biphenyl (RfD = 0.5 mg/kg/day; IRIS CASRN 92-52-4, 2013) and approximate the maximum residue limit set for their use as pesticides in fruit (0.01 mg/kg; EU Pesticides database, 2016), their presence in the SB is undesirable. It has been proved that in the concentrations above 0.05 mg/l they cause off-flavours in soft drinks. To detect contaminated SB and prevent undesirable taste and aroma changes we propose including sensory evaluation of SB (e.g. in the form of a 0.2 g/l aqueous solution) to raw material inspection processes. The presented GC-O method was found to be more suitable for control practices, because thanks to the pre-concentration and chromatographic separation, it can detect lower concentrations in aromatised beverages. Additional corrective measures to eliminate

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the incidence of off-flavours include revising the required specifications for the purity of SB.

References

Amerine M.A., Pangborn R.M., Roessler E.B. (2013): The sense of taste – XV. Sodium benzoate. In: Anson M.L., Mrak E.M., Chichester C.O. (eds): Principles of Sensory Evaluation of Food. Academic Press: 114–115.

Bleibaum R.N., Stone H., Tan T., Labreche S., Saint-Martin E., Isz S. (2002): Comparison of sensory and consumer results with electronic nose and tongue sensors for apple juices. Food Quality and Preference, 13: 409–422.

Brattoli M., Cisternino E., Dambruoso P.R., de Gennaro G., Giungato P., Mazzone A., Palmisani J., Tutino M. (2013): Gas chromatography analysis with olfactometric detection (GC-O) as a useful methodology for chemical characterization of odorous compounds. Sensors (Basel), 13: 16759–16800.

Čížková H., Voldřich M., Ševčík R., Pivoňka J. (2009): Off-flavour defects of packed waters and soft drinks. Czech Journal of Food Sciences, 27: S379–S381.

Grégrová A., Čížková H., Rajchl A., Šnebergrová J., Voldřich M. (2014): Průzkum obsahu benzenu v nealkoholických nápojích. Kvasný průmysl, 60: 8–14.

Horsáková I., Voldřich M., Čeřovský M., Sedláčková P., Šicnerová P., Ulbrich P. (2009): *Asaia* sp. as a bacterium decaying the packaged still fruit beverages. Czech Journal of Food Sciences, 27: S366–S368.

Högnadóttir Á., Rouseff R.L. (2003): Identification of aroma active compounds in orange essence oil using gas chromatography-olfactometry and gas chromatography-mass spectrometry. Journal of Chromatography A, 998(1–2): 201–211.

Hundley J.G., Nathan F.: Manufacture of benzoic acid from toluene. Patent US 3210416 A, 1965.

Juvonen R., Virkajärvi V., Priha O., Laitila A. (2011): Microbiological spoilage and safety risks in non-beer beverages. VTT Tiedotteita – Research Notes, 2599: 107. doi: 10.13140/RG.2.1.3166.8562

Leitnerová D., Panovská Z., Váchová A., Boháčková B. (2010): Posouzení čichového vnímání prostřednictvím komerčně dostupné sady pachů. Chemické listy, 104: 861–866.

Ma X., Deng J., Fang J., Shanaiah N., Smiley E., Dietrich A.M. (2016): Identification and characterization of phenylacetonitrile as a nitrogenous disinfection byproduct derived from chlorination of phenylalanine in drinking water. Water Research, 102: 202–210.

Murnane S.S., Lehocky A.H., Owens P.D. (2013): Odor Thresholds from Chemicals with Establshed Health Standards. 2nd Ed. Virginia, American Industrial Hygiene Association: 90.

Pang X., Guo X., Qin Z., Yao Y., Hu X., Wu J. (2012): Identification of aroma-active compounds in Jiashi muskmelon juice by GC-O-MS and OAV calculation. Journal of Agricultural and Food Chemistry, 60: 4179–4185.

Perez-Cacho P.R., Rouseff R. (2008): Processing and storage effects on orange juice aroma: a review. Journal of Agricultural and Food Chemistry, 56: 9785–9796.

Plutowska B., Wardencki W. (2008): Application of gas chromatography–olfactometry (GC–O) in analysis and quality assessment of alcoholic beverages – a review. Food Chemistry, 107: 449–463.

Ridgway K., Lalljie S.P.D., Smith R.M. (2009): Analysis of food taints and off-flavours – a review. Food Additives and Contaminants, 27: 146–168.

Rouseff R., Naim M. (2007): Citrus Flavor Stability. In: Berger R.G. (ed.): Flavours and Fragrances. New York, Springer: 117–134.

Šístková I., Haubeltová A., Pohůnek V., Čížková H. (2017): Identifikace senzoricky aktivních látek minerálních vod s citronovou příchutí pomocí plynové chromatografie s olfaktometrickým detektorem. Chemické listy, 111: 343–348.

Van Den Dool H., Kratz P.D. (1963): A generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography. Journal of Chromatography A, 11: 463–471.

Wei W., Li J., Huang L. (2017): Discrimination of producing areas of *Astragalus membranaceus* using electronic nose and UHPLC-PDA combined with chemometrics. Czech Journal of Food Sciences, 35: 40–47.

Widén H., Leufvén A., Nielsen T. (2005): Identification of chemicals, possibly originating from misuse of refillable PET bottles, responsible for consumer complaints about off-odours in water and soft drinks. Food Additives and Contaminants, 22: 681–692

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