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Contamination from the environment as a source of bottled water off-odour: a case study

Kontaminace z okolí jako příčina přípachu balené pramenité vody: případová studie

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The aim of this work is to devise a methodology and identify the cause of off-odour in bottled water. For initial screening, sensory analysis and gas chromatography with mass spectrometry and olfactometric detector were used. Selected compounds were then analyzed and quantified by selective ion monitoring. 2,4-decadienal, a fatty acid oxidation product, was identified as the source of off-odour. It is produced during frying and it can diffuse through the packaging material into the product. Its concentration in three analyzed samples ranged from $0.15 \mu\text{g/l}$ to $3.1 \mu\text{g/l}$. Since its detection threshold is $1 \mu\text{g/l}$, improper storage conditions can decrease the sensory quality of bottled water.

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Cílem práce bylo navrhnout metodiku identifikace příčiny přípachu balené pramenité vody. Nejdříve byla provedena senzoričká analýza a necílený screening přítomných těkavých látek metodou plynové chromatografie s hmotnostním a olfaktometrickým detektorem. Pro následující cílenou analýzu byla zvolena metoda výběru charakteristických iontů zaměřená na kvantifikaci vytipovaných senzoričsky aktivních látek. Z těchto látek byl za původce přípachu označen 2,4-dekadienal, produkt oxidace mastných kyselin vznikající například při smažení, který může difundovat přes obal do výrobku. Koncentrace 2,4-dekadienu se ve 3 analyzovaných vzorcích pohybovaly od $0,15 \mu\text{g/l}$ do $3,1 \mu\text{g/l}$. Jelikož práh detekce vůně této látky je $1 \mu\text{g/l}$, existuje riziko, že nevhodné podmínky skladování sníží smyslovou kvalitu balené vody.

Keywords: quality, beverage, water, off-odour, SPME-GC/MS/O, volatile compounds**Klíčová slova:** kvalita, nápoj, voda, přípach, SPME-GC/MS/O, těkavé látky

1 INTRODUCTION

Sensory characteristics like appearance, taste and aroma are the basic parameters to evaluate the quality of the product, based on which the consumer decides whether to buy it again. According to the World Health Organization drinking water should be free of any tastes and odours, that would be objectionable to the majority of consumers (WHO, 2017). In the Czech Republic the quality requirements for bottled water are regulated by Decree No. 275/2004 (Ministry of Health, 2004), which says that bottled water may not contain the causative or indicating organisms of diseases and exhibit any organoleptic defects. Since water does not contain any flavoring ingredients, consumers will easily detect any sensory defects.

Sensory defects of bottled water may be caused by:

1) Microbial contamination

Metabolic products of algae, cyanobacteria or bacteria – earth-smelling geosmin and 2-methylisoborneol (2-MIB) described as musty are the most commonly reported flavors and odours in the water industry. Their production has been confirmed in bacteria of the genus *Actinomyces*, *Lysobacter*, *Streptomyces*, some amoebae and several genus of mycobacteria (Zaitlin and Watson, 2006). These substances have been identified in drinking water from surface water sources and the traditional water treatment (coagulation, flocculation, filtration through a sand filter) will not remove them (Chen et al., 2013).

Musty odour is considered one of the hardest to remove and it is also caused by, for example 2,4,6-trichloranisol (2,4,6-TCA) (also responsible for cork off-odour in wine), 2-isopropyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine which are metabolites of actinomycetes and soil bacteria (e.g., mycobacteria). 2,4,6-TCA is formed by the microbiological methylation of halophenols (which are produced as chlorination by-products) during water treatment or during transport (Zhou et al., 2017).

Off-odours can also be caused by sulfuric bacteria. They produce sulfur-smelling substances (also described as sweet or pungent) such as hydrogen sulphide, methylmercaptan and dimethylpolysulphides in the drinking water distribution network. These substances can be produced either as degradation products of sulfuric amino acids or by biogenic production of inorganic sulphides which are subsequently methylated with a specific thiol S-methyltransferase in the presence of organic methyl donors (e.g., L-Met, dimethylsulfoxide and S-methylmethionine) (Kristiana et al., 2010).

2) Contamination with chemicals from the production process

Disinfectants used in water treatment can be sensorially active or react with chemicals present in untreated water and create smelly by-products. (Dietrich, 2006). For example, if a water that contains traces of phenol or natural organic substances (eg. humic and fulvic acids) is chlorinated, chlorophenols can be produced and give the drink a medicinal flavor (Young et al., 1996). Chlorine and chloramines can also react with oil-based tube lubricants to produce rancid flavors. Although chlorine is an odorant itself, it can be used to remove fish odors. Ozone on the other hand removes the earthy and musty odor of geosmine and 2-MIB, but can give rise to fruity-smelling aldehyde compounds (Rogers, 2001).

Anti-corrosion and lubricating agents, such as those used for blow molding machines for polyethylene terephthalate (PET) bottles, can also be a source of contamination (Čížková et al., 2009). The sources of other contaminating sensory active substances (phthalates, alkyl phenols, antioxidants, UV stabilizers, lubricants and carbonyl compounds) in bottled water can be bottle caps, pipelines, disinfectants, water treatment and bottling or even environmental pollution (Guart et al., 2014).

3) Contamination with chemicals from packaging material

If residual ozone remains in the treated water after ozonization, it can react with the polymeric packaging materials to form sensory active substances. High density polyethylene bottles and polypropylene caps give rise to a range of C4 to C9 aldehydes. PET bottles release cyclic PET oligomers and erucamide. The most important substances causing undesirable off-odours include carbonyl compounds: acetaldehyde (sweet, fruity), formaldehyde (pungent), acetone (ethereal, apple) or methylglyoxal (sweet, caramel) and nona-

nal (fatty, potato) (Nawrocki et al., 2002). C4-C7 aldehydes and other substances, for example butanol, 3-hexanone, 2-hexanone, and 3-heptanone may be formed from the ethyl vinyl acetate layers. These volatile substances are infamous because they cause off-odour and off-taste in bottled water and their detection threshold is very low (Song et al., 2003).

4) Improper storage conditions

Gases and volatile organic compounds can migrate through the polymeric packaging material into the product and degrade it. In general, the more crystalline the polymer is, the better the barrier properties it has. Of the conventional packaging materials, the greatest migration rate will be for polyethylene and the smallest for PET (Bairrie, 2003). Closures which are permeable to oxygen may transmit organic substances which have a negative impact on the sensory properties of the product (Lopes et al., 2012).

Packaging material may also be a source of contaminants due to improper storage conditions. Since all polymers degrade due to external factors such as solar radiation and temperature, the storage of PET bottles under uncontrolled conditions can be a source of organic pollutants. Among other compounds, generated either after exposure to UV radiation, or due to long storage periods are (Z)-non-2-enal, (E)-non-2-enal, (E)-dec-2-enal, (tr)-4,5-epoxy-(E)-dec-2-enal, having a greasy or plastic flavor, and oct-1-ene-3-one, γ -octalactone (sweet or coconut), vanillin and ethyl vanillin. These carbonyl compounds are attributed to so-called „sunlight“ off-odour in mineral waters (Strube et al., 2009).

The aim of the submitted work was to propose a methodology for identification and determination of the cause of fatty off-odour of bottled spring water. Sensory analysis was performed by a panel of tested assessors using a triangular test. Subsequently, the target compounds were identified after isolation by solid phase microextraction in conjunction with a gas chromatograph with a mass spectrometer and an olfactometric detector (SPME-GC/MS-O). In addition, the discovered substances, responsible for off-odour, were quantitated and the limit of detection was determined.

2. MATERIALS AND METHODS

2.1 Samples and chemicals

3 samples of spring water were received in January 2017 (number 1 – comparative, numbers 2 and 3 – claimed). Samples were in 0.5 liter glass bottles, sealed with a crown cap with a Granulen liner without PVC, with a minimum shelf life of 12 months. The chemicals used were - denatured ethanol, nonanal (95%, Sigma-Aldrich) and 2,4-decadienal (2,4-DD) (> 94%, Sigma-Aldrich).

2.2 Sensory analysis of samples

A triangular test was used to detect odours and determine the difference in odour in water samples. Samples were presented to the assessors in combination with distilled water in clear, transparent plastic cups. Analysis was attended by 11 assessors, each tested two sets of each sample. The preparation procedure and the course of the test is specified by the standard ČSN EN ISO 4120 (2009). The evaluation was performed at significance levels of 99 and 95%.

2.3 Sample screening with SPME-GC-MS/O

5g of samples were weighed into 10 ml glass vials and closed with caps with septa. Isolation was performed using SPME 50/30 μ m DVB/CAR/PDMS fiber, Stableflex. The preincubation was 60 seconds, the incubation temperature was 50 °C, the extraction lasted 25 minutes, and the desorption was 4 minutes. Aroma analysis was performed on a 7890B gas chromatograph with a DB-5MS column (30 m x 250 μ m x 0.25 μ m), a 5977 A MSD mass detector and a GC Sampler 80 from Agilent Technologies and a Joint Analytical System olfactometric detector. The injector temperature was 240 °C, using split 1:1. The helium carrier gas flow was maintained at 1.4 ml/min. The temperature mode was set at 60 °C for 2 minutes with a gradual rise in temperature of 10 °C per minute to 290 °C. The eluent was divided at the column outlet in a 1:1 ratio into a mass detector and via a transport tube heated to 180 °C to an olfactometric detector. The temperature in the mass detector was 230 °C and 150 °C in the quadrupole. Total analysis time was 25 minutes. The interpretation was carried out from the Total Ion Chromatogram (TIC), which includes the entire range of masses detected at each time of the analysis, and provides overall information on the measured sample. The NIST integrated library was used for identification.

The detection of the aroma active substances took place using a glass olfactometric extension without air humidification and a posterior intensity method (scale 1-5) was used. Four trained assessors, non-smokers aged 23-25 years, evaluated each sample 2 times (Šístková, et al., 2017).

2.4 The quantification of selected substances

The external standard method was used for the quantification of nonanal and 2,4-DD, the samples were analyzed by SIM (selective ion monitoring) with a selection of characteristic ions: 98 (quantified) and 57 (confirmation) for nonanal, 81 (quantified) and 41 (confirmation) for 2,4-DD, the other chromatographic conditions are specified in chapter 2.3. The standards were dissolved in 3 ml of ethanol before dilution, then transferred to a 1 liter flask and filled up with distilled water. Then the appropriate concentrations (0.03, 0.15, 0.3, 3, 6, 9 μ g/l) were prepared by diluting into volumetric flasks.

2.5 Limit of detection of nonanal and 2,4-DD

The limit of detection was first tested using the triangular test specified in chapter 2.2. and then on the olfactometer under the conditions specified in chapter 2.3.

The initial concentration used for the triangular test was 1 μ g/l for both substances, and the number of evaluators was 16 (24-45 years, 13 women non-smokers and 3 males out of which 1 was a smoker). Other concentrations used for nonanal were 20 and 100 μ g/l, and 10 assessors participated in the analysis.

The limit of detection from the triangle test (1 and 100 μ g/l) was used as a starting point for olfactometric analysis. Subsequent concentrations used were 25, 10 and 5 μ g/l for nonanal and 0.50 and 0.75 μ g/l for 2,4-DD. The analysis was carried out by four trained assessors, non-smokers aged 23-25. Women were preferred because they are reported to be more sensitive to some fragrances than men (Čejka et al., 2018).

3 RESULTS AND DISCUSSION

3.1 Sensory analysis

The expected atypical odour of the samples was first confirmed by a triangular test. The difference between sample 2 and distilled water was statistically significant at the 99% level of significance (the difference was recognized by 19 evaluators out of 22). For sample 3, this applies at a level of significance of 95% (16 evaluators out of 22). No difference was found in sample 1 against distilled water (the difference was correctly identified by 11 evaluators out of 22).

3.2 Sample screening

Substances responsible for the off-odour of bottled water were identified by the SPME-GC-MS/O method. Most sensory active sub-

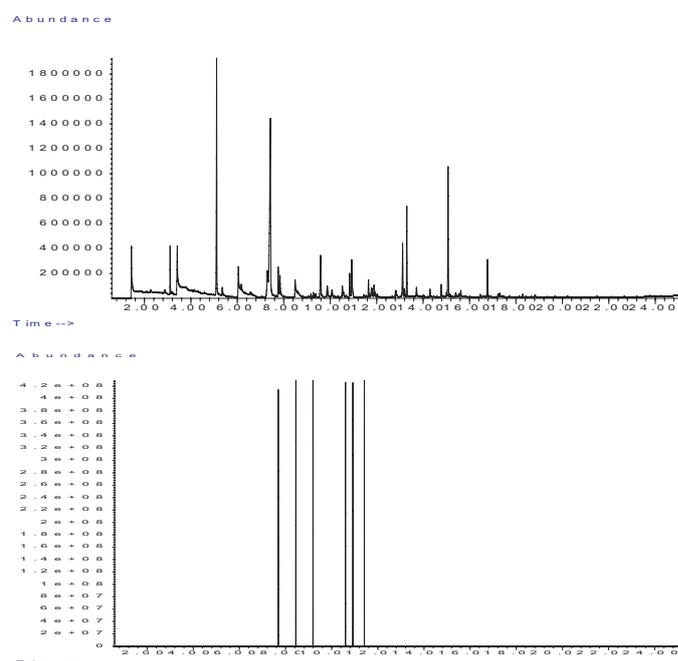


Fig. 1 Recorded substances by assessor A in sample No. 3

stances had a very low intensity (1-2 of 5 points) and the agreement between the four evaluators was very low, identification with the NIST mass spectral library was not possible (they are probably monoterpenes, alcohols and aldehydes). Fig. 1 shows the chromatogram and the aromagram of the 6 sensory active substances recorded by the assessor A for sample No. 3. The measured values indicated that the undesirable odor was most likely caused by nonanal (recorded by three evaluators) and 2,4-DD (recorded by all evaluators) which have been described as sweet, fatty, greasy and rancid, and which intensity of perception has been repeatedly high (3-4 of 5 points). These substances are the degradation products of fatty acids oxidation (oleic, linoleic, arachidonic) (Tavassoli-Kafrani et al., 2016), originating, for example, from frying (Frankel, 2014). Further analysis was focused on these substances.

3.3 Quantification of substances likely to be responsible for off-odour

For the quantification of nonanal and 2,4-DD, the selective ion monitoring (SIM) method with the selection of characteristic ions was chosen to increase sensitivity. The limit of quantification (LOQ) for both substances was set at 0.3 µg/l and the linearity in the observed concentration range of 0.3 to 9 µg/l was confirmed. The detection limit (LOD) was determined at 0.15 µg/l and was significantly affected by a) the probable matrix effect and the transmission of the monitored substances between the analyzes that not even the optimization steps completely excluded, b) the limited options to confirm the identity of the compounds in trace concentrations using a characteristic weight ratio spectrum. The repeatability of the measurement at a concentration level of 0.3 µg/l, calculated as the relative standard deviation of 5 repetitive measurements, was 15%, which seems acceptable with respect to the isolation method (SPME) and the very low concentration level. The results of the assay are shown in Table 1.

Table 1 Nonanal a 2,4-DD concentration in the samples

Sample	Concentration (µg/l)	
	Nonanal	2,4-DD
1	<LOQ	<LOD
2	<LOQ	3.13
3	0.31	0.88

LOD... limit of detection, LOQ... limit of quantification

3.4 Limit of detection for nonanal and 2,4-decadienal

The responses from both tests for the determination of the limit of detection are summarized in Table 2. The detection limit from the triangular test was determined as 1 µg/l for 2,4-DD and 20-100 µg/l for nonanal. The detection limit for these substances in water reported in the literature (for nonanal 1-98 µg/l for 2,4-DD 0.07-7.3 µg/l) (Bartosz, 2013; Belitz, H-D et al., 2009; Plotto et al., 2004) is very heterogeneous and indicates a significant influence of the analysis conditions and individual sensitivity of the evaluators, which also corresponds with our results. To determine the exact detection limit for nonanal a significantly greater number of evaluators would be required. The detection threshold for 2,4-DD was set at 0.75 µg/l and 5-10 µg/l for nonanal. The reason why these thresholds are lower than the ones from the triangular test analysis is that instrumental analysis involves concentrating the sample on the SPME fiber and so the substances can be detected at lower concentrations.

Table 2 The responses from the triangular experiment and olfactometric analysis

Nonanal (µg/l)	detected	not detected	2,4-DD (µg/l)	detected	not detected
Triangular test					
1	1	15	1	8	8
20	2	8	x	x	x
100	7	3	x	x	x
Olfactometric analysis					
25	8	0	1	7	1
10	7	1	0.75	4	4
5	1	7	0.5	0	8

4 CONCLUSIONS

The results confirm that 2,4-decadienal was in samples 2 and 3 above the odor threshold which was determined olfactometrically (0.75 µg/l) and for sample 2 above the odour threshold determined by the triangular test (1 µg/l) and was therefore the cause of an undesirable off-odour. Nonanal was found in all samples, but with respect to a significantly higher detection threshold (1-98 µg/l) (Czerny et al., 2008) does not represent a hazard in the found concentrations (0.15 to 0.31 µg/l) and according to literature (Sieg et al., 2009) occurs as a common contaminant. The olfactometric assay is challenging for the selection and training of the assessors, and there is no forced choice unlike in the triangle test. Fewer assessors took part in the analysis, but even a panel this small is able to identify substances at very low concentrations. Therefore olfactometry is a sensitive method that is more objective than the triangular test and an alignment with mass detector enables effective identification of undesirable off-odours.

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