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Short communication

Benzaldehyde in cherry flavour as a precursor of benzene formation in beverages

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ABSTRACT

During sampling and analysis of alcohol-free beverages for food control purposes, a comparably high contamination of benzene (up to 4.6 μg/L) has been detected in cherry-flavoured products, even when they were not preserved using benzoic acid (which is a known precursor of benzene formation). There has been some speculation in the literature that formation may occur from benzaldehyde, which is contained in natural and artificial cherry flavours. In this study, model experiments were able to confirm that benzaldehyde does indeed degrade to benzene under heating conditions, and especially in the presence of ascorbic acid. Analysis of a large collective of authentic beverages from the market ($n = 170$) further confirmed that benzene content is significantly correlated to the presence of benzaldehyde ($r = 0.61$, $p < 0.0001$). In the case of cherry flavoured beverages, industrial best practices should include monitoring for benzene. Formulations containing either benzoic acid or benzaldehyde in combination with ascorbic acid should be avoided.

1. Introduction

Benzene is one of the compounds with the highest level of evidence that it may cause cancer in humans (Steinbrenner, Löbell-Behrends, Reusch, Kuballa, & Lachenmeier, 2010). For example, it was classified into group 1 by the International Agency for Research on Cancer (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012). For this reason, levels of benzene in foods "shall be kept as low as can reasonably be achieved by following good practices" (ALARA principle) according to the EU regulation on food contaminants (European Council, 1993), and for some food groups such as drinking water maximum limits have been implemented by law; for example a maximum limit of 1.0 μg/L is demanded by the EU drinking water directive (European Council, 1998). There may be two different mechanisms causing the occurrence of benzene in foods and beverages: the products may either be (often inadvertently) contaminated from external sources containing benzene (such as petrol), or benzene may be formed intrinsically as a heat-induced contaminant from various natural or artificial precursors found in the foods (Lachenmeier, Kuballa, et al., 2010; Lachenmeier, Steinbrenner, Löbell-Behrends, Reusch, & Kuballa, 2010; Salviano dos Santos, Medeiros Salgado, Guedes Torres, & Signori Pereira, 2015). While the first mechanism is well manageable and has not led to large problems except for isolated cases, e.g., due to contaminated carbon dioxide used in beverage production (Wu, Lin, Fan, Dong, & Chen, 2006), the second mechanism was of considerable concern when benzene contamination was regularly detected in alcohol-free beverages with levels of up to 23 μg/L (Cao, Casey, Seaman, Tague, & Becalski, 2007; Gardner & Lawrence, 1993; Lachenmeier, Reusch, Sproll, Schoeberl, & Kuballa, 2008; McNeal, Nyman, Diachenko, & Hollifield, 1993; Nyman et al., 2008; Page, Conacher, Weber, & Lacroix, 1992). The source of this contamination, benzoic acid used as an additive for preservation, was quickly

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detected. Benzoic acid may deteriorate to considerable contents of benzene, especially when used in combination with ascorbic acid (Salviano dos Santos et al., 2015). Industry guidelines were established suggesting the replacement of benzoic acid with other preservatives, specifically when used in combination with ascorbic acid (either naturally contained or used as an antioxidant additive) (Salviano dos Santos et al., 2015). It was therefore surprising when consumer magazines reported the occurrence of comparably high contents of benzene in beverages not preserved with benzoic acid. A concentration of 4.6 μg/L was detected in a cherry beverage (Stiftung Warentest, 2013b), which led to a larger survey of cherry beverages ($n = 30$), from which 6 were found positive, with levels between 0.3 and 2.5 μg/L of benzene. As the benzene levels were roughly correlated to the occurrence of benzaldehyde (9.5–135 mg/L), this led to the hypothesis that benzaldehyde may be the precursor of benzene formation (Stiftung Warentest, 2013a). The scientific literature offers only limited evidence to corroborate this hypothesis. McNeal et al. (1993) showed in a model solution that 74 μg/kg benzene may form from benzaldehyde at 0.04% (w/w) in water in the presence of 0.025% ascorbic acid. Nyman, Wamer, Begley, Diachenko, and Perfetti (2010) detected the formation of 0.3 μg/kg benzene in a cherry flavoured drink after storage for 14 days at 40 °C. The benzaldehyde content of the drink was 54 mg/kg.

This study was conducted to provide evidence into the potential mechanism of benzene formation in beverages from benzaldehyde by model experiments and correlation analysis of data from a large market survey.

2. Materials and methods

2.1. Survey of cherry-flavoured beverages

Between January and March 2014, 170 samples submitted to the CVUA Karlsruhe were analysed for benzene, benzaldehyde and benzoic acid. As part of official food control, our institute is the central beverage control laboratory in Baden-Württemberg, a German federal state with a population of approximately 10.6 million. The sampling has been done by local authorities directly at food producers or retail trade. The samples have been randomly selected and collected by government food inspectors, as our institute has requested only the food group and sample number to collect but not specific brands. For this survey, we requested the food groups "soft drinks with cherry flavour", and "mineral or table water flavoured with cherry". Some additional samples (about 10%) were directly purchased over the internet or in wholesale stores in Karlsruhe, Germany. In total, we received 72 cherry-flavoured samples. Additionally, 98 soft drinks and flavoured water samples with other flavours except cherry were analysed for comparison. Non-parametric statistical comparisons (Mann–Whitney tests) between groups of samples were conducted, using the software package Stat Tools for Excel Version 5.5.0 (Palisade Corporation, Ithaca, NY). Linear correlation analysis was conducted using Origin V.7.5 (Originlab, Northampton, MA).

2.2. Benzene formation from pure benzaldehyde (model experiment)

In order to evaluate the possible benzene formation capability of benzaldehyde in aqueous solution with a minimum amount of experiments, a D-optimal experimental design was used. The D-optimal algorithm chooses an ideal subset of all possible combinations and significantly reduces the number of required experiments compared to standard design types. To simulate the matrix of cherry-flavoured beverages, a citrate buffer at pH 3.5 was chosen as the medium (the pH was chosen because alcohol-free drinks typically have pH values between 3 and 4). Benzaldehyde and/or ascorbic acid were added at a concentration of 75 mg/L or 90 mg/L, respectively. The heating temperature was studied at three levels (unheated i.e. room temperature at about 20 °C, 60 °C, and 100 °C). In the case of the heated samples, the heating time was varied at two levels (2 h, and 24 h). The experiments were conducted with 10 mL of liquid directly in the headspace vials that were measured afterwards without opening to avoid any loss of benzene. In total, 23 experiments were conducted in duplicate ($n = 46$). The experimental designs and calculations were done using the Software Package Design Expert V7.0.0 (Stat-Ease Inc., Minneapolis, MN).

2.3. Chemical analysis

Benzene was analysed using a validated headspace-gas chromatography/mass spectrometry (HS-GC/MS) procedure with a deuterated internal standard, previously described in detail (Lachenmeier et al., 2010; Lachenmeier et al., 2008). The quantification was conducted in the selected ion monitoring (SIM) mode; for benzene: m/z 78 as target ion and m/z 77 as qualifier ion, and for benzene-d_6 as internal standard: m/z 84 as target ion and m/z 82 as qualifier ion. Determined according to the German norm DIN 32645, the limit of detection was 0.03 μg/L and the limit of quantitation was 0.09 μg/L.

Benzaldehyde was analysed using a separate HS-GC/MS procedure. For this, 1 mL of sample was mixed with 4 mL of water, 100 μL of internal standard (benzaldehyde-d_6, 40 μg/mL in methanol) and 100 μL of methanol. Using 100 μL of 25% KOH, the solution was adjusted to approximately pH 10 to neutralise the carbonic acid contained in some samples. The quantification was conducted in the selected ion monitoring (SIM) mode; for benzaldehyde: m/z 106 as target ion and m/z 105 and m/z 77 as qualifier ions, and for benzaldehyde-d_6 as internal standard: m/z 112 as target ion and m/z 110 as qualifier ion. The limit of detection was 0.5 mg/L and the limit of quantitation was 1.7 mg/L.

Finally, benzoic acid was determined using HPLC according to the German reference method (Anon, 1984). The limit of detection was 0.5 mg/L and the limit of quantitation was 1.5 mg/L.

3. Results

The results of the model experiment are shown in Fig. 1. The analysis of variance (ANOVA) for the response surface quadratic model implied that the model is significant ($p < 0.0001$, F-value 23.18) with a coefficient of determination (r^2) of 0.9040. The model parameters were judged as adequate to navigate the design space. The following model terms were significant: heating time ($p = 0.0002$), benzaldehyde ($p < 0.0001$), ascorbic acid ($p < 0.0001$) and temperature ($p < 0.0001$). The following interactions between model terms were significant: heating time-benzaldehyde ($p = 0.0024$), heating time-ascorbic acid ($p = 0.0027$), benzaldehyde-ascorbic acid ($p < 0.0001$), benzaldehyde-temperature ($p < 0.0001$), ascorbic acid-temperature ($p = 0.0002$). It is therefore obvious that benzene may be formed from benzaldehyde under heating, and to a greater extent in the presence of ascorbic acid.

The results of the survey of benzene, benzaldehyde and benzoic acid in 170 beverages are shown in Table 1. The average benzene concentration in cherry-flavoured beverages was 0.18 μg/L, while the value in beverages with other flavours was 0.08 μg/L. A significant linear relation between benzaldehyde and benzene concentrations was detected ($r = 0.6122$, $p < 0.0001$) (Fig. 2), but not between benzoic acid and benzene concentrations ($r = 0.1016$, $p = 0.2338$). When the samples were sorted in benzene negative