SHORT COMMUNICATION

THE MECHANISM OF SPOILAGE OF A BOTTLED SOFT DRINK LEADING TO HEALTH PROBLEM OF CONSUMER

Lenka Mayerová[1], František Kožíšek[1], Dana Baudišová[1], Kateřina Klánová[2], Barbora Hlaváčková[3]
[1]Centre for Health and the Environment, National Institute of Public Health, Prague, Czech Republic
[2]Centre of Toxicology and Health Safety, National Institute of Public Health, Prague, Czech Republic
[3]Regional Public Health Authority of the Central Bohemian Region, Prague, Czech Republic

SUMMARY

The case of spoilage of flavoured and sweetened soft drink occurred in the Czech Republic in 2019. After drinking it, the consumer was admitted to a hospital with sickness. The spoilage was caused by mould and an odorous substance, 1,3-pentadiene. The mechanism of food spoilage, with the formation of 1,3-pentadiene arising from the decarboxylation of sorbic acid and mould growth, is described. This could be the second case history reported worldwide of an allergic reaction to penicillin explaining how penicillin might get into the beverage. We hypothesise three possible causes of the health problem experienced with allergic reaction to penicillin or other mycotoxin produced by *Penicillium* mould as the most probable one.

Key words: soft drink, 1,3-pentadiene, mould, penicillin, allergy

Address for correspondence: L. Mayerová, National Institute of Public Health, Šrobárova 49/48, 100 00 Prague 10, Czech Republic. E-mail: lenka.mayerova@szu.cz

https://doi.org/10.21101/cejph.a7388

INTRODUCTION

There are a number of reasons why food is spoiled. Sometimes, a single cause, and sometimes, a combination or chain of causes may lead to food spoilage. Signs of food spoilage and consequences of spoiled food consumption can also vary widely. As food spoilage often manifests itself by changed organoleptic characteristics, humans have effective tools (sense organs) at their disposal to identify such defects. That is why, after a critical sensory evaluation, a spoiled food product is often thrown straight away as garbage without any further attempt to identify which microorganisms or chemicals caused the spoilage. In this short communication, we present a case report of sensory barrier failure, not unique but interesting because the ingestion of a visibly spoiled food product resulted in consequent health problems.

The case of spoilage of soft drink (flavoured and sweetened bottled water) occurred in the Czech Republic in 2019. The consumer of middle age reported to have bought two 1.5 L bottles of still, strawberry flavoured soft drink and to have had a glass (approx. 200 mL) of it to swallow a painkiller after lunch. The consumer drank the beverage immediately after having poured it from the full freshly opened bottle in a glass, without noticing any unpleasant taste or smell. Shortly after that, she was feeling dizzy, had a headache, and was feeling faint. At the same time, she noticed an unusual smell (strong smell of nail polish remover, solvent or gasoil) coming from the opened bottle with the beverage. On closer examination, she noticed whitish structures floating in the bottle. It was for the first time she bought this type of soft drink and so was unaware of how it should actually taste.

As the symptoms did not resolve, she called an emergency ambulance and was taken to a regional hospital where she reported to have penicillin allergy (experienced in early childhood as rash or dermatitis). Additional symptoms like scratching and burning in the throat and then also in the stomach appeared later in the hospital. Nevertheless, no treatment was needed after admission, she was only observed and then discharged, without any symptoms, on the following day.

MATERIALS AND METHODS

On the initiative of the police, the case was investigated by the Regional Public Health Authority. They collected three types of samples of this soft drink and ordered an analysis at the regional Institute of Public Health Ústí nad Labem (IPHUL): sample 1 (from the bottle opened by the complainant), sample 2 (unopened bottle with the factory sealed cap purchased by the complainant, and six sealed bottles of this drink officially obtained from the same shop shortly after this case arose) and sample 3 (six sealed bottles taken about one month after the incident at the request of the retail chain).

All samples were subjected to a sensory assessment and chemical and microbiological analysis. A basic analysis was supplemented with a special chemical analysis using the solid phase microextraction gas chromatography/mass spectrometry (SPME

GC-MS) in the full scan mode. The analysis of 1,3-pentadiene by SPME GC-MS was performed according to the IPHUL standard operating procedure (SOP 346), which serves to characterize the environmental samples containing mixtures of volatile organic compounds (1). The anion-active surfactants were analysed according to EN 903:1993 (2). The fungi (moulds) were analysed at the National Institute of Public Health (NIPH) according to EN ISO 6222:1999 (3) with a subsequent verification according to EN ISO 21527-1:2014 (4). The sensory assessment was performed by a sensory panel according to EN ISO 8586:2012 (5) in both IPHUL and NIPH laboratories.

Since there are no specific legislative quality requirements for soft drinks, the measured values (concentration) of chemical indicators were evaluated with regard to legislative requirements for bottled spring water, which was used to produce this drink (Czech Decree No. 275/2004 Coll.).

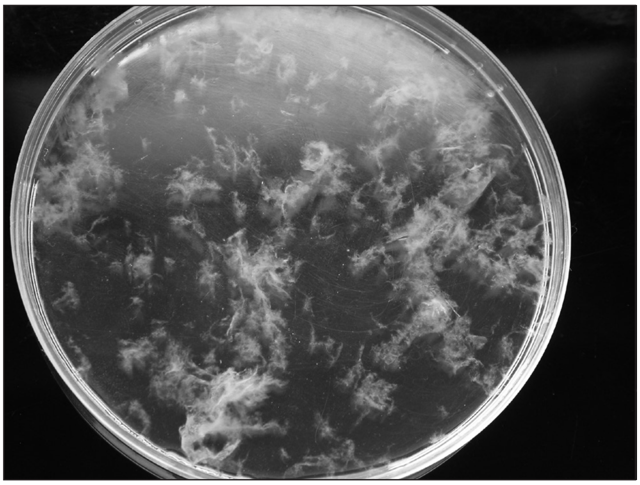

Fig. 1. *Sample 3 – flavoured and sweetened bottled water with whitish structures after 1 weekup.*

RESULTS

The sensory assessment of sample 1 found the following result: a clear liquid containing clumping structures which was foamier after shaking than would be expected for non-carbonated soft drink. After the bottle was opened, a strong odour of solvent (of nail polish remover or gasoil) or benzine was detected. The container was a soft transparent PET bottle without any visible damage.

The microbiological analysis of faecal indicators, *P. aeruginosa*, sporulated sulphite-reducing anaerobes, and colony counts of samples 2 and 3 showed zero colony forming units (CFU) in all parameters. The analysis of sample 1 showed positive finding of moulds only (result was not quantified) as more detailed identification was not performed for this sample.

The chemical analysis showed a low pH value of 3.0 and the presence of anion-active surfactants 0.06 mg L^{-1} for all samples (samples 1, 2, 3). The rest of parameters (conductivity, turbidity and non-polar extractive substance in sample 1 and about 50 parameters in samples 2 and 3 did not show any non-compliance with bottled spring water limit values. Even if the limit of 0.02 mg L^{-1} for anion-active surfactants specified by Czech Decree No. 275/2004 for bottled spring water, used in the production of the drink tested, was exceeded, a single intake of the altered drink does not pose a health risk to humans. Moulds were detected by colony count techniques in sample 1 and 1,3-pentadiene were detected by the SPME GC-MS as full scan (monitoring provides a qualitative picture of the composition of the sample).

No 1,3-pentadiene or any other contamination caused by an organic compound was found in sample 2, only butanoic and propanoic acid esters were detected, which are flavourings added to the strawberry flavoured spring water. Also, no moulds were detected in this sample.

Sample 3 was positive for yeasts and moulds at a count of 6,600 CFU mL^{-1} in one of the six bottles sampled. A pooled sample from all six bottles was analysed and turned out to be positive for 1,3-pentadiene.

Despite the fact that no sensory defect was evident in sample 3 at the time of opening the bottle, after about one week of its storage in the fridge, whitish structures and unacceptable odour were also formed, and so the sample was handed over to the NIPH probably in the same condition as when the drink was consumed by the complainant (Fig. 1). Fungi of the genus *Penicillium* (*Penicillium* spp.) were detected in sample 3 (Fig. 2).

Fig. 2. *Sample 3 – Penicillium spp.*

The presence of the organic compound 1,3-pentadiene in the sample from the complainant and in sample 3 along with the detection of moulds and visible growth of whitish structures point to microbiological degradation of the soft drink.

DISCUSSION

Preservatives are routinely used to prevent such food spoilage. These are chemicals that kill microorganisms by inhibiting the enzyme systems vital for their growth (6). Sorbic acid salts (potassium sorbate and calcium sorbate) and sorbic acid itself are commonly used food preservatives. They act as a quite effective inhibitor of the growth of a variety of moulds, yeasts, and bacteria and can be used for the preservation of beverages, selected types of cheese, some types of dough, bakery products, fats, sauces, egg products, and other foods (6–8).

Sorbic acid salts are more commonly used because of their higher water solubility and ease of handling than the acid itself. The most widely used sorbic acid salt is potassium sorbate: CH3-CH=CH-CH=CH-COOK. Potassium sorbate works best at a pH of <5 but is less active at a pH of 6.5. It inhibits the growth of gram-positive and gram-negative bacteria. Potassium sorbate is an antimicrobial with low toxicity that does not kill the cell and only inhibits its growth. It is used in soft drinks, bakery products and syrups (6, 9).

The spoiled soft drink contained, as shown on the label, (spring) water, sugar, potassium sorbate, citric acid, and natural aroma (base 580718, exact composition not found); thus, there may have been hydrolysis to give sorbic acid and potassium dihydrogen citrate with subsequent decarboxylation of sorbic acid to 1,3-pentadiene in the presence of moulds (Fig. 3). Nevertheless, balance is rather shifted to the side of the initial ingredients, which means that the presence of potassium sorbate and citric acid predominates. This can be explained by the fact that citric acid and sorbic acid have similar pKa (4–5); consequently, the driving force for hydrolysis is low. However, potassium sorbate as such can decarboxylate under heating directly to 1,3-pentadiene without the need for hydrolysis into sorbic acid.

Kinderlerer et al. (10) state that sorbic acid can be decarboxylated by moulds to produce 1,3-pentadiene, which no longer has antifungal activity. Decarboxylation is a chemical reaction in which a carboxyl group is lost from a parent molecule as carbon dioxide (CO_2) (Fig. 4). Some moulds are able to decarboxylate sorbic acid to produce 1,3-pentadiene according to the following reaction (Fig. 4) (6, 8).

Sokołowska et al. (11) aimed to identify the off-odour compounds in samples of strawberry-flavoured soft drink preserved with potassium sorbate and sodium benzoate. The mould isolated from this sample was identified as *Penicillium corylophilum*. The drink was also evaluated for the ability to form undesirable compounds. The results revealed the presence of 1,4-pentadiene and benzaldehyde in the tested samples.

A direct link between microbiological spoilage and the formation of 1,3-pentadiene was demonstrated by Cuhra (8), who observed that the microbial degradation of sorbic acid was accompanied by intensive microbial growth, often visible even to the naked eye as whitish structures or turbidity; thus, the spoilage can also be detected by sight. The author carried out an experiment, inoculating mould colonies isolated from spoiled strawberry syrup into microbiologically uncontaminated syrup with the same composition where no 1,3-pentadiene was present and no signs of spoilage were evident. After several days, 1,3-pentadiene was detected, and its concentration was progressively increasing, along with the decreasing content of sorbic acid and presence of other negative signs of spoilage, such as odour, visible growth of mycelium and emergence of whitish structures. The mould isolated from samples

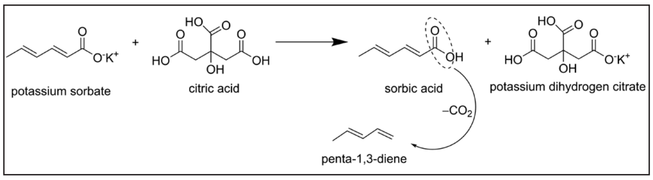

Fig. 3. *Formation of 1,3-pentadiene in the presence of moulds.*

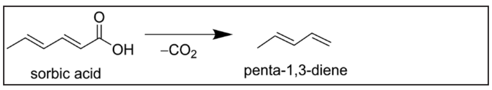

Fig. 4. *Decarboxylation sorbic acid by moulds to produce 1,3-pentadiene.*

of the strawberry syrups containing 1,3-pentadiene was identified as *Penicillium corylophilum*. Interestingly, co-degradation of some other flavour compounds was observed in these strawberry syrups, which made already negative sensory properties even worse.

Later, this author noticed a number of other, completely analogous cases in other soft drinks: peach or lemon flavoured iced tea, non-carbonated tropical flavoured multivitamin lemonade, and a range of other drinks. In all cases, microorganisms were detected in the samples, but, surprisingly, apart from moulds, yeasts or bacteria also caused degradation. Sometimes, a whole batch of the product was contaminated, and sometimes, this was only the case with some bottles (e.g. one bottle out of six to ten), which makes it difficult to identify the contaminated batch or to trace the contaminated bottles in practice. While in the former case (the whole batch contaminated), the contamination seems to result from the failure to comply with hygienic standards in the production of the product, in the latter case (individual bottles contaminated), an unambiguous cause could not be identified. The common denominator of the problems in the beverage sector was the fact that all contaminated samples originated from PET or other plastic bottles and that all sampled bottles contained non-carbonated soft drinks.

The most probable explanation is that when several pallets with PET bottles are stacked on top of each other, when pressed by the weight of the upper layer, some caps may let air in, thus allowing microbial contamination to enter.

Given that fungi of the genus *Penicillium* (*Penicillium* spp.) were detected in sample 3, it can be hypothesised that the reason for the complainant's admission to hospital might be penicillin allergy, i.e., reaction of the immune system to penicillin or penicillin-like substance present in the drink. Common signs and symptoms of penicillin allergy, typically triggered by penicillin-based antibiotics, include itchy palms and feet, skin redness, hives, rash, difficulty breathing, difficulty swallowing, difficulty speaking, low blood pressure, and fast and irregular heartbeat. These symptoms can even lead to a loss of consciousness and failure of vital functions (12).

Although some experts regard as unlikely that a person who is allergic to *Penicillium* mould or penicillin would have an allergic reaction when drinking bottled water or soft drink with *Penicillium* mould present in it (e.g., US Army Institute of Public Health) (13), one such case report is available in the literature. Wicher and Reisman (14) described a case history of a woman with extreme sensitivity to penicillin who experienced anaphylactic reaction (itching eyes and palms followed by generalized urticaria) shortly after ingestion of 90 mL of carbonated grapefruit juice soft drink. They did not find the fungi in the drink, but they proved the presence of penicillin activity with a biological test (inhibition of growth of *Staphylococcus aureus*); unfortunately, they did not identify the source of penicillin in the soft drink. It might be questionable if the symptoms experienced by the woman in our case were really relating to penicillin allergy as the most common symptom of allergic reactions elicited by penicillin in milk is dermatitis (pruritus, rash, urticaria); however, less common symptoms include joint swelling, angioedema, recurrent fever, lymph-node enlargement, and headache (15). The woman had a combination of systemic or general and local symptoms, which may both fall under anaphylactic reaction to penicillin, while it is highly improbable that such symptoms would just be caused by an unpleasant taste or the odorous substance 1,3-pentadiene, which remained unnoticed by the patient while drinking the beverage. Although 1,3-pentadiene in higher concentration (theoretically > 10%, practically > 30%) may be skin and eye irritable, its concentration in respective bottled water was < 1%, i.e., not relevant for any hazardous classification.

Micromycetes (fungi) produce a battery of enzymes able to break down a variety of organic material. They are adapted to conditions where most bacteria are no longer capable of metabolism as they proliferate even at a low pH. They can grow at very low temperatures (up to minus 10 ºC), but do not survive short heating to 70–75 ºC (16, 17). The most important food spoilage-related genera of toxigenic fungi are *Aspergillus*, *Fusarium*, and *Penicillium*. Other mycotoxin producers are the genera *Trichoderma* and *Alternaria* (18, 19).

The genus *Penicillium* includes about 150 species. These species form colonies with a large number of yellow-green to blue-green conidia. They are found on various foods and other surfaces as green, velvety to powdery growths. The edges of these colonies free of spores are white. Mycotoxins produced by the genus *Penicillium* can be found in foods such as cereals, corn, rice, fruits (apples, pears, and strawberries), vegetables (potatoes and tomatoes), peanuts, pistachio nuts, meat products, or cheeses. Some species may trigger allergy. The most important species are *Penicillium expansum*, *Penicillium chrysogenum*, *Penicillium aurantiogriseum*, or *Penicillium roqueforti* (20). The *Penicillium* species detected in the soft drink was not identified by us. Neither did we attempt to analyse penicillin or penicillin-like substances in the soft drink. That is why we have to admit there is no direct evidence that health problems experienced by the woman were really caused by penicillin allergy. Other hypothetical explanations would include simple qualmishness from swallowing the food with heavy sensory defect, or toxic/allergic reaction elicited by other unidentified chemical substance present in the drink. However, according to our opinion, hypothesis of allergy triggered by penicillin or other mycotoxin produced by *Penicillium* mould seems to be the most probable of those three possible causes.

CONCLUSION

Food can get spoiled due to a single cause or chain of causes as was the case with this soft drink. It contained, among others, potassium sorbate which can decarboxylate under heating and in the presence of moulds directly to 1,3-pentadiene, responsible for degradation of sensory qualities of the product. The producers of soft drinks, especially flavoured and sweetened ones, should monitor possible sources of fungal contamination during the production, distribution and storage. It is vital to check the safety of all ingredients, compliance with the process hygiene criteria on the filling line, bottle and bottle cap safety and tightness, and adequate handling during the distribution and storage.

A case where a person is admitted to hospital after drinking a soft drink is rather rare but may occur, as has just been shown. Particularly if there is a spoiled food product with fungal growth (anyone can be allergic to any species of mould) and if sensory barrier fails. We hypothesise this might be probably the second case history reported of an allergic reaction to penicillin from a non-alcoholic beverage and the first report to explain how penicillin might get into the beverage.

All public should be educated to avoid consumption of the food with strange or unusual smell, taste, or appearance.

Acknowledgements

This article was supported by the Ministry of Health within the project MZ ČR – RVO (National Institute of Public Health – SZÚ, ID 75010330).

Conflict of Interests

None declared

REFERENCES

1. SOP 346. SPME GC-MS method, volatile organic compounds (screening): manual at regional Institute of Public Health Ústí nad Labem. Ústí nad Labem: Institute of Public Health Ústí nad Labem; 2019. (In Czech.)
2. EN 903. Water quality - Determination of anionic surfactants by measurement of the methylene blue index (MBAS). Brussels: European Committee for Standardization; 1993.
3. ISO 6222. Water quality - Enumeration of culturable micro-organisms - Colony count by inoculation in a nutrient agar culture medium. Geneva: International Organization for Standardization; 1999.
4. ISO 21527-1. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds - Part 1: Colony count technique in products with water activity greater than 095. Geneva: International Organization for Standardization; 2008.
5. EN ISO 8586. Sensory analysis - General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors. Brussels: European Committee for Standardization; 2014.
6. Brul S, Coote P. Preservative agents in foods: mode of action and microbial resistance mechanisms. Int J Food Microbiol. 1999 Sep 15;50(1-2):1-17.
7. Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. Off J Eur Union. 2008 Dec 31;51(L 354):16-33.
8. Cuhra P. Sorbic acid - assistant or potential threat? Výživa a potraviny. 2009;64(3):66-7. (In Czech.)
9. Guynot ME, Marín S, Sanchis V, Ramos AJ. An attempt to optimize potassium sorbate use to preserve low pH (4.5-5.5) intermediate moisture bakery products by modelling Eurotium spp., Aspergillus spp. and Penicillium corylophilum growth. Int J Food Microbiol. 2005;101(2):169-77.
10. Kinderlerer JL, Hatton PV. Fungal metabolites of sorbic acid. Food Addit Contam. 1990;7(5):657-69.
11. Sokołowska B, Połaska M, Dekowska A, Woźniak Ł, Roszko M. Degradation of preservatives with the formation of off-odor volatile compounds - the case of strawberry-flavored bottled water. Beverages. 2020;6(4):67. doi: 10.3390/beverages6040067.
12. Vyles D, Adams J, Chiu A, Simpson P, Nimmer M, Brousseau DC. Allergy testing in children with low-risk penicillin allergy symptoms. Pediatrics. 2017 Aug;140(2):e20170471. doi: 10.1542/peds.2017-0471.
13. U. S. Army Institute of Public Health. Mold in military bottled water supplies. Fact sheet 21-017-0814. Aberdeen Proving Ground: APHC.
14. Wicher K, Reisman RE. Anaphylactic reaction to penicillin (or penicillin-like substance) in a soft drink. J Allergy Clin Immunol. 1980;66(2):155-7.
15. Dewdney JM, Edwards RG. Penicillin hypersensitivity - is milk a significant hazard?: a review. J R Soc Med. 1984;77(10):866-77.
16. Northolt MD, Bullerman LB. Prevention of mold growth and toxin production through control of environmental conditions. J Food Prot. 1982;45(6):519-26.
17. Magan N, Olsen M, editors. Mycotoxins in food: detection and control. Cambridge: Woodhead Publishing; 2004.
18. Pitt JI. Toxigenic fungi and mycotoxins. Br Med Bull. 2000;56(1):184-92.
19. Blumenthal CZ. Production of toxic metabolites in Aspergillus niger, Aspergillus oryzae, and Trichoderma reesei: justification of mycotoxin testing in food grade enzyme preparations derived from the three fungi. Regul Toxicol Pharmacol. 2004 Apr;39(2):214-28.
20. Laich F, Fierro F, Martín JF. Production of penicillin by fungi growing on food products: identification of a complete penicillin gene cluster in Penicillium griseofulvum and a truncated cluster in Penicillium verrucosum. Appl Environ Microbiol. 2002;68(3):1211-9.

Received March 14, 2022
Accepted in revised form October 6, 2022