Shelf life of bottled water – field conditions in Hungary

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Summary
Professional soldiers and firefighters are deployed to deal with the consequences of various disasters, where their supply is carried out under field conditions. This study investigated whether improperly stored bottled drinking water can change its quality and pose a biological hazard. Microbiological quality of 20 samples of bottled mineral water produced in Hungary, including 10 uncarbonated, 5 carbonated and 5 flavoured mineral water samples, was investigated under field conditions. Culturable microorganisms were enumerated by ISO 6222:1999; coliforms and *Escherichia coli* by ISO 9308-1:2000; *Pseudomonas aeruginosa* by ISO 16266:2006; *Enterococcus* spp. by ISO 7899-2:2000; and *Clostridium perfringens* by ISO 14189:2013. In six cases among uncarbonated water samples, the aerobic colony counts exceeded the standard value. Furthermore, coliforms and *P. aeruginosa* were detected in three cases. However, in carbonated and flavoured mineral water, no samples of unacceptable bacteriological quality were observed, as their pH value was significantly lower and that probably did not favour proliferation of bacteria. Due to their acidic condition, carbonated and flavoured mineral water appears to be less vulnerable to microbiological contamination under field conditions. During flood damage remediation, it is advisable to perform the supply of intervention units with carbonated and flavoured mineral water to avoid infection.

Keywords
drinking water; biological hazard; water storage; bottled mineral water

Although natural mineral waters have been consumed since Roman times, only the 20th century has seen emergence of the natural mineral water industry and drinking of these products on a large scale as an alternative to tap water and non-alcoholic beverages [1]. Professional soldiers, firefighters, emergency managers, members of non-governmental organizations and volunteers who carry out field-based remediation tasks are increasingly confronted with biological hazards during disaster relief, in particular during flood remedies [2–4]. Floods in Hungary are fairly common [5, 6] and, according to the practical experience of the authors, it is obvious that the activities, including restoration, can last several weeks, during which food and drinking water must be provided in place. Previously, several papers investigated the aspects of field conditions in supply management [7–12] as well as microbiological analysis of bottled drinking water [13–15]. Our present study was placed at the intersection of these two topics, as we examined bottled drinking water under field conditions.

The concept of natural mineral water is defined by Directive 2009/54/EC [16] as microbiologically wholesome water originating in an underground water table or deposit, and emerging from a spring tapped from one or more natural or bore exits. It may not be subjected to any treatment aside from the separation of unstable constituents and the elimination, introduction or re-introduction of carbon dioxide. Any treatment likely to change the viable colony counts of the natural mineral water is strongly prohibited. The total colony

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counts, often referred to as heterotrophic plate counts (*HPC*), measured within 12 h after bottling, should not exceed 100 CFU·ml^{-1} after incubation for 72 h at 20–22 °C, and 20 CFU·ml^{-1} after 24 h at 37 °C. Directive 2009/54/EC [16] also specifies three conditions that during marketing, natural mineral water shall be free from (a) parasites and pathogenic microorganisms, (b) *Pseudomonas aeruginosa*, enterococci, *Escherichia coli* and other coliforms in any 250 ml sample examined, and (c) sporulating sulfite-reducing anaerobes in any 50 ml sample examined.

Hungary is rich in mineral and spring waters [17], as well as the country's national supply chain is secured continuously [18]. The consumption of bottled mineral water and other drinking water is increasing year by year, confirming the importance of our research. Most of these waters are commercially available in bottled form, but in recent years they are most commonly available in polyethylene terephthalate (PET) bottles. Even during flood protection, PET bottled water is transported to the units in most cases. That is why we used these for our research subjects.

Due to the limitations of the present research, the entire supply chain could not be investigated, so only the biological risks of drinking water supply were addressed. During recent remediation, we observed that the supply of drinking water for the units was performed via bottled drinking water, mineral water and flavoured drinking water (hereinafter referred as "drinking water"). Bottled drinking water is a special foodstuff with guaranteed quality. Only mineral water or spring water can be bottled, which has been officially certified by the competent authority of the country of origin based on test results [19]. In addition, even manufacturing companies guarantee quality consistency through their own chemical, analytical and biological assays. It is obvious that these bottled drinking waters are stored in open areas for a shorter or longer period before being placed on the market, exposed to weather conditions. During disaster relief, such as flood protection, storage also takes place in an open area where temperature fluctuations (in particular in summer) and varying intensity of sunlight can trigger changes in the quality of drinking water [13, 20]. Among these changes, only microbiological factors are investigated for bottled drinking water that we selected from the commercial traffic. The examined parameters were determined according to the applicable Hungarian legislation and standards [21]. The major factors and practices that influence the changes in microbial populations and contamination of bottled mineral waters are well studied and understood. In addition, the current legislation in Hungary and in most European countries requires that food business operators put in place, implement and maintain a permanent procedure or procedure based on the principles of hazard analysis and critical control points (HACCP).

MATERIALS AND METHODS

Samples

The investigated PET-bottled water was bought in grocery stores in Hungary, choosing the brands that are mostly supplied during the flood control. A total of 20 samples were taken, including 10 non-carbonated, 5 carbonated and 5 flavoured drinking water. As a first step, the bottled drinking water was stored in an open area for a period of 2 weeks under field conditions. We have paid attention to testing all bottles before the expiration date specified by the manufacturer. Prior to microbiological testing, each sample was numbered, non-carbonated drinking water with 1–10, carbonated drinking water with 11–15, and flavoured drinking water with 16–20.

pH measurement

The pH value was measured using a certified WTW 3430 Sen Tix 940-3 type measuring equipment (Aktivit, Budapest, Hungary).

Microbiological analysis

Our microbiological tests were carried out in the accredited water testing laboratory of Pannon Víz (Győr, Hungary). We examined the microbiological parameters determined in the Ministerial decree No. 65/2004 (IV.27.) [22] on the rules of bottling and marketing of natural mineral water, spring water, drinking water, drinking water enriched with minerals and flavoured water, and in Government decree No. 201/2001 (X.25.) [21] on drinking water quality requirements and inspection procedures. Microbiological test parameters and permitted limits are shown in Tab. 1. It should be noted that the aerobic and anaerobic colony counts determined at 22 °C and 37 °C are applicable to samples taken and tested 12 h after bottling. The other limits apply throughout the entire product's shelf life. All laboratory tests were performed according to the requirements of the relevant standardization.

Culturable microorganisms were enumerated according to ISO 6222:1999 [23]. Coliforms and *Escherichia coli* were enumerated according to ISO 9308-1:2000 [24]. *Pseudomonas aeruginosa* was enumerated according to ISO 16266:2006

[25]. Enterococci was enumerated according to ISO 7899-2:2000 [26]. *Clostridium perfringens* was enumerated according to ISO 14189:2013 [27]. For filtering, a 0.22 μm pore diameter EZ-HAWG 474 membrane filter (Merck, Darmstadt, Germany) was used. All culture media used in microbiological analyses were from Biolab, Budapest, Hungary.

RESULTS AND DISCUSSION

Data on pH values of the samples are shown in Tab. 2. Reviewing them, it can be seen that the values of non-carbonated drinking water were around 7, of carbonated drinking water around 4 and of flavoured drinking water between 3 and 4.

The numbers of bacteriologically unacceptable samples among non-carbonated drinking water samples are summarized in Tab. 3. It can be observed that the aerobic colony counts were unfavourable. Of the 10 non-carbonated drinking water samples tested, six were found to have exceeded the permissible level of psychrophilic aerobic colony counts. Six samples did not comply with legal requirements regarding mesophilic aerobic colony counts. Coliforms were detected in two samples and *P. aeruginosa* in one sample. Enterococci, *E. coli* and mesophilic sulfite-reducing bacteria were not present in any of the samples. In the case of non-carbonated drinking water samples analysed, 60 % were non-compliant. For some microbiological tests, the sum of the values shown did not equal the total number of hygienically non-compliant samples because there were samples for which several test parameters were objected. Tab. 4 summarizes the results of the microbiological tests for non-carbonated water.

Analysing the results, it can be stated that in total 6 samples developed bacteria during the incubation period.

The test results for carbonated drinking water are given in Tab. 3. The bacteriological quality of carbonated drinking water was 100 % compliant with the legal requirements. This was probably due to the fact that the bacteria do not develop in an acidic medium. This was also supported by the pH values shown in Tab. 2.

The results of microbiological analysis of the flavoured drinking water samples are shown in Tab. 3. These also had significantly better hygienic quality than the non-carbonated drinking water. Each of the five samples examined complied with the legal requirements. This result can also be explained by the fact that the tested bacteria do not proliferate at low pH of the medium, which was formed by the substances added to the natural mineral water (e.g. sweeteners, citric acid, preservatives, texturizing agents, artificial dyes). The pH values of flavoured drinking water were in many cases lower than the pH values of carbonated drinking water (Tab. 2).

It can be concluded that 60 % of the non-carbonated drinking water tested did not meet the criteria prescribed by the relevant legislation. We also found that, in most of the samples, high bacterial counts posed a quality risk. Storage under

Tab. 1. Microbiological test parameters and permitted limits [21, 22].

Microbiological parameter	Accepted limit [CFU·ml^{-1}]
Aerobic colony count at 22 °C	100
Aerobic colony count at 37 °C	20
Coliform bacteria	0*
Enterococcus spp.	0*
Escherichia coli	0*
Pseudomonas aeruginosa	0*
Mesophilic sulfite-reducing clostridia	0*
Parasitic and pathogenic microbes	0

* – Presence of bacteria is tested in 250 ml (mesophilic sulfite-reducing clostridia in 50 ml).

Tab. 2. pH values of tested water samples.

Serial number of the sample	pH value
Non-carbonated drinking water	
1	7.51
2	7.68
3	7.50
4	7.48
5	7.65
6	7.55
7	7.70
8	7.65
9	7.42
10	7.54
Carbonated drinking water	
11	4.02
12	3.96
13	3.90
14	4.10
15	4.25
Flavoured drinking water	
16	3.40
17	3.38
18	3.18
19	3.25
20	3.32

Tab. 3. Drinking water samples of unacceptable bacteriological quality.

Microbiological parameter	Number of samples of unacceptable quality		
	Uncarbonated drinking water (n = 10)	Carbonated drinking water (n = 5)	Flavoured drinking water (n = 5)
Aerobic colony count at 22 °C	6	0	0
Aerobic colony count at 37 °C	6	0	0
Coliform bacteria	2	0	0
Enterococcus spp.	0	0	0
Escherichia coli	0	0	0
Pseudomonas aeruginosa	1	0	0
Mesophilic sulfite-reducing clostridia	0	0	0
Sum of unacceptable samples	6	0	0

Tab. 4. Aggregate microbiological test results for non-carbonated drinking water.

Sample	1	2	3	4	5	6	7	8	9	10
Number of colonies at 22 °C [CFU·ml^{-1}]	> 100	0	> 100	> 100	0	> 100	> 100	0	0	> 100
Number of colonies at 37 °C [CFU·ml^{-1}]	> 20	0	> 20	> 20	0	> 20	> 20	0	0	> 20
Presence in sample										
Coliform bacteria	–	–	–	+	–	–	+	–	–	–
Enterococcus spp.	–	–	–	–	–	–	–	–	–	–
Escherichia coli	–	–	–	–	–	–	–	–	–	–
Pseudomonas aeruginosa	–	–	–	–	–	–	+	–	–	–
Mesophilic sulfite-reducing clostridia	–	–	–	–	–	–	–	–	–	–

Presence of coliform bacteria was detected in Sample 4 and Sample 7 in volume of 250 ml, presence of *P. aeruginosa* was detected at Sample 7 in volume of 250 ml.

inappropriate conditions, particularly in sunlight and at high temperatures, is expected to promote bacterial growth in bottled, non-carbonated drinking water. This is also supported by the research conducted in Taiwan [15], which investigated the microbiological condition of bottled mineral water. In that study, total heterotrophic cell counts were above the legal limit, but no coliforms or fecal streptococci were detected. The bottled drinking water was then stored at 25 °C and re-examined for microbiological status, which revealed that the microbial counts increased rapidly to 10^4–10^5 CFU ml^{-1} under these conditions. It is important to note that, according to the Decree 65/2004 (IV.27.) [22], the limit for mesophilic and psychrophilic aerobic colony counts is for the first 12 h after filling and not for the entire shelf life. The tested bottled drinking water was commercially available, so the tests could be performed well after 12 h of filling, so that poor storage conditions played a role in poor quality samples. It is regrettable that a significant proportion (60 %) of the bottled non-carbonated drinking water samples did not comply with current bacteriological standards. The most serious problem in the samples was the high colony counts but, in two cases, coliforms and, in one, case *P. aeruginosa* was detected. We found that, in contrast to non-carbonated mineral water, there was no bacteriological difference in carbonated and flavoured mineral water compared to the legal requirements. Bottled mineral water may become contaminated with factory equipment (filters, tanks, pipelines) during the process, but bacterial proliferation is greatly facilitated by inadequate storage conditions.

Bottled mineral water of lower pH is apparently less vulnerable to bacterial proliferation. Lower pH of drinking water should not pose problems regarding human consumption as it is tolerated by the human organism. The pH value of stomach fluids is in the range of 1.0–3.5, so drinking water enriched with CO_2 (pH 3.3–4.02 determined in this study) should not harm the body in this respect [28].

CONCLUSIONS

In the present study, microbiological parameters of bottled uncarbonated, carbonated and flavoured mineral water produced in Hungary were examined under field conditions. Culturable microorganisms, coliforms, *E. coli*, *P. aeruginosa*, enterococci and *C. perfringens* were enumer-

ated according to international standards. In six cases from among uncarbonated water samples, the aerobic colony counts exceeded the standard value. Furthermore, in three cases, coliforms and *P. aeruginosa* were detected. However, in carbonated and flavoured mineral water samples, no unacceptable bacteriological quality was observed.

It would be advisable to use carbonated or flavoured mineral water when supplying drinking water to the units performing remediation activities, as they do not pose a microbiological risk to consumers, given the storage conditions in the field. Furthermore, based on our research experience, we recommend bottled drinking water companies to consider lowering the pH of non-carbonated mineral waters, as a change in storage conditions would not pose a microbiological risk to consumers. With our research, we wanted to highlight the importance of the topic and we recommend considering the results as well as suggestions presented in this article in everyday practice.

REFERENCES

1. Stickler, D. J.: The microbiology of bottled natural mineral waters. Journal of the Royal Society of Health, *109*, 1989, pp. 118–124. DOI: 10.1177/146642408910900404.
2. Kuti, R. – Grósz, Z.: Biológiai eredetű veszélyhelyzetek kezelése, előtérben a mentesítési feladatok. (Management of biological emergencies, with a focus on relief tasks.) Hadmérnök, *11*, 2016, pp. 125–132. ISSN: 1788-1929. <http://www.hadmernok.hu/161_13_kutir_gz.pdf> In Hungarian.
3. Taylor, J. – Lai, K. – Davies, M. – Clifton, D. – Ridley, I. – Biddulph, P.: Flood management: Prediction of microbial contamination in large-scale floods in urban environments. Environment International, *37*, 2011, pp. 1019–1029. DOI: 10.1016/j.envint.2011.03.015.
4. Wölz, J. – Fleig, M. – Schulze, T. – Maletz, S. – Lübcke-von Varel, U. – Reifferscheid, G. – Kühlers, D. – Braunbeck, T. – Brack, W. – Hollert, H.: Impact of contaminants bound to suspended particulate matter in the context of flood events. Journal of Soils and Sediments, *10*, 2010, pp. 1174–1185. DOI: 10.1007/s11368-010-0262-y.
5. Albright, E. A.: Policy change and learning in response to extreme flood events in Hungary: An advocacy coalition approach. Policy Studies Journal, *39*, 2011, pp. 485–511. DOI: 10.1111/j.1541-0072.2011.00418.x.
6. Vari, A.: Public involvement in flood risk management in Hungary. Journal of Risk Research, *5*, 2002, pp. 211–224. DOI: 10.1080/13669870110042606.
7. Dwivedi, Y. K. – Shareef, M. A. – Mukerji, B. – Rana, N. P. – Kapoor, K. K.: Involvement in emergency supply chain for disaster management: a cognitive dissonance perspective. International Journal of Production Research, *56*, 2018, pp. 6758–6773. DOI: 10.1080/00207543.2017.1378958.
8. King, N. – Roberts, D. E. – Edwards, J. S. A. – Morizen, R. D. – Askew, E. W.: Cold-weather field feeding: An overview. Military Medicine, *159*, 1994, pp. 121–126. DOI: 10.1093/milmed/159.2.121.
9. Pujawan, I. N. – Kurniati, N. – Wessiani, N. A.: Supply chain management for Disaster Relief Operations: principles and case studies. International Journal of Logistics Systems and Management, *5*, 2009, pp. 679–692. DOI: 10.1504/IJLSM.2009.024797.
10. Spieler, P. – Petersen, H.: Maintenance of water and food quality in the stabilization forces area of operations: German surveillance laboratories and the problem of expired field rations. Military Medicine, *165*, 2000, pp. 346–350. DOI: 10.1093/milmed/165.5.346.
11. Teixeira, P. – Cunha, J. – Albano, H. – Ramalho, R. – Gibbs, P.: Evaluation of survival patterns and cellular injury of *Pseudomonas aeruginosa* in different bottled waters stored under various conditions. Journal of Food Safety, *21*, 2001, pp. 167–180. DOI: 10.1111/j.1745-4565.2001.tb00316.x.
12. Xu, X. – Zhang, S. – Wu, Q. – Zhang, J. – Li, F. – Cheng, J.: Development and application of a Loop-Mediated Isothermal Amplification (LAMP) method for rapid and sensitive detection of *Enterococcus faecalis* in drinking water. Journal of Food Safety, *34*, 2014, pp. 103–110. DOI: 10.1111/jfs.12101.
13. Biedermann-Brem, S. – Grob, K.: Release of bisphenol A from polycarbonate baby bottles: water hardness as the most relevant factor. European Food Research and Technology, *228*, 2009, pp. 679–684. DOI: 10.1007/s00217-008-0978-8.
14. Fujikawa, H. – Wauke, T. – Kusunoki, J. – Noguchi, Y. – Takahashi, Y. – Ohta, K. – Itoh, T.: Contamination of microbial foreign bodies in bottled mineral water in Tokyo, Japan. Journal of Applied Microbiology, *82*, 1997, pp. 287–291. DOI: 10.1046/j.1365-2672.1997.00353.x.
15. Tsai, G. J. – Yu, S. C.: Microbiological evaluation of bottled uncarbonated mineral water in Taiwan. International Journal of Food Microbiology, *37*, 1997, pp. 137–143. DOI: 10.1016/s0168-1605(97)00065-2.
16. Directive 2009/54/EC of the European Parliament and of the Council of 18 June 2009 on the exploitation and marketing of natural mineral waters (Recast). Official Journal of European Communities, *52*, 2009, L164, pp. 45–58. <https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32009L0054>
17. Borszéki, B. G.: Ásványvizek, gyógyvizek. (Mineral waters, medicinal waters.) Budapest : Self-published, 1998. ISBN: 9635505833009. In Hungarian.
18. Barreto, S. – Bártfai, B. – Engloner, A. – Liptay, Á. – Madarász, T. – Vargha, M.: Water in Hungary In: Hungarian Academy of Sciences [online]. Budapest : Hungarian Academy of Sciences [September 28, 2020]. <https://mta.hu/data/dokumentumok/Viztudomanyi%20Program/Water_in_Hungary_2017_07_20.pdf>
19. Sipos, L.: Ásványvízfogyasztási szokások elemzése

és ásványvizek érzékszervi vizsgálata. (Analysis of mineral water consumption habits and sensory examination of mineral waters.) [Dissertation thesis.] Budapest : Corvinus University of Budapest, 2009. <http://phd.lib.uni-corvinus.hu/360/1/sipos_laszlo.pdf> In Hungarian.

20. Zhang, S. – Xu, X. – Wu, Q. – Zhang, J.: Rapid and sensitive detection of Pseudomonas aeruginosa in bottled water by loop-mediated isothermal amplification. European Food Research and Technology, *236*, 2013, pp. 209–215. DOI: 10.1007/s00217-012-1876-7.
21. 201/2001. (X. 25.) Korm. rendelet az ivóvíz minőségi követelményeiről és az ellenőrzés rendjéről (Government Decree No. 201/2001 on Drinking Water Quality Requirements and Inspection Procedures.) Magyar Közlöny, *118*, 2001, pp. 8188–8236. ISSN: 0076-2407. In Hungarian.
22. 65/2004. (IV. 27.) FVM-ESzCsM-GKM együttes rendelet a természetes ásványvíz, a forrásvíz, az ivóvíz, az ásványi anyaggal dúsított ivóvíz és az ízesített víz palackozásának és forgalomba hozatalának szabályairól. (Ministerial decree No. 65/2004 on the rules of bottling and marketing of natural mineral water, spring water, drinking water, drinking water enriched with minerals and flavored water.) Magyar Közlöny, *57*, 2004, pp. 5916–5923. ISSN: 0076-2407. In Hungarian.
23. ISO 6222:1999. Water quality – Enumeration of culturable micro-organisms – Colony count by inoculation in a nutrient agar culture medium. Geneva : International Organization for Standardization, 1999.
24. ISO 9308-1:2000. Water quality – Detection and enumeration of *Escherichia coli* and coliform bacteria – Part 1: Membrane filtration method. Geneva : International Organization for Standardization, 2000.
25. ISO 16266:2006. Water quality – Detection and enumeration of *Pseudomonas aeruginosa* – Method by membrane filtration. Geneva : International Organization for Standardization, 2006.
26. ISO 7899-2:2000. Water quality – Detection and enumeration of intestinal enterococci – Part 2: Membrane filtration method. Geneva : International Organization for Standardization, 2000.
27. ISO 14189:2013. Water quality – Enumeration of *Clostridium perfringens* – Method using membrane filtration. Geneva : International Organization for Standardization, 2013.
28. O'May, G. – Reynolds, N. – Macfarlane, G. T.: Effect of pH on an in vitro model of gastric microbiota in enteral nutrition patients. Applied Environmental Microbiology, *71*, 2005, pp. 4777–4783. DOI: 10.1128/AEM.71.8.4777-4783.2005.

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