Bottled Water Microbiological Quality in Estonia

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Abstract
Nowadays people often prefer to buy bottled water instead of drinking the tap water. Stores are full of different bottled waters and studies have demonstrated that people tend to consider the bottled water to have high quality (Ward et al 2009, Akpinar et al 2014). The aim of this research was to find out the compliance of bottled water sold in Estonia with the established microbiological normatives. The study included bottled drinking water available in retail stores. A total of 63 bottles of water from 21 different sale items were analyzed. These 21 items included also carbonized waters and flavoured waters. All different waters (21 bottles) were analysed for coliforms, *E. coli*, *Enterococcus sp* and *Pseudomonas aeruginosa* (filtration method, cfu/250 mL), and all 63 bottles were analysed for total bacterial count at 22 °C and 37 °C (pour-plate technique, cfu/1mL).
No coliforms, *E. coli*, *Enterococcus sp* nor *P. aeruginosa* were detected in the analyzed waters. Several deviations were noted for total microbial count: 14 bottles out of 63 had bacteria level higher than the established normatives allow, thus 22.22% of bottles were microbiologically contaminated. Study results suggest that carbonization of bottled water has an inhibitory effect on the total bacterial count; sweetened waters richer in bacterial nutrients did not show higher bacterial counts. Experiments of storage conditions showed that only waters with initially low microbial counts can be stored for longer time, in bottles with initially high microbial counts have risk for increase in time.
Keywords: bottled water, microbiological quality

1. Introduction

According to the European Federation of Bottled Waters 2017 market report 48% of sold non-alcoholic beverages was packed water (bottled water).
Bottled water quality must meet the requirements of Drinking water directive 98/83/EC. Directive requires to monitor and test regularly microbiological, chemical and indicator parameters. Microbiological parameters are specified for *E. coli* 250 mL, enterococci 250 mL, *P. aeruginosa* 250 mL, colony count 1 mL at 22 °C and 37 °C. Previous studies have shown that microbiological parameters of bottled drinking water do not always meet the requirements. Microorganisms have been found more often in non-carbonated waters than carbonated waters (Varga 2011, Korzeniewska et al 2005). Duranceau et al (2012) demonstrated that multiplication of heterotrophic bacteria might become a problem during storage of drinking water, the temperatures averaging 35 ºC created the maximum counts of heterotrophic bacteria up of 4000 cfu/mL.
The aim of current research was to find out the compliance of bottled water sold in Estonia to the Drinking water directive 98/83/EC, especially as tap water in Estonia has mostly very high quality, but based on previous study 16.5% of responders have said that they consider bottled water to have higher quality than tap water (Pastak 2017).

2. Sample Collection and Analysis

The study included bottled drinking water available in retail stores of Estonia. A total of 63 bottles of drinking water were analysed. This included 21 different sales items: 7 carbonated waters, 7 non-carbonated waters and 7 flavoured waters. Three samples (bottles) of each item were collected and analysed.
As presence of coliforms including *E. coli*, enterococci and *P.aeruginosa* was not expected in the bottled drinking water, these microbes were investigated only from one of the three bottles of the same kind. For investigation of coliforms including *E. coli* (ISO 9308-1:2014), enterococci (ISO 7899-2:2002) and *P. aeruginosa* membrane filtration of 250 mL was used, one of the filters was put on chromogenic coliform agar (Biolife) and another filter to Slanetz-Bartley medium (Biolife). As *P. aeruginosa* grows also on the chromogenic coliform agar, additional medium was not used. Both agars were incubated at 37 °C 24±3 hours. There were only very few colonies growing on these media after incubation and none of those could be confirmed to be as bacteria we were looking for.
Standard ISO 6222: 1999 with pour plate technique was used to enumerate colonies at 22 °C and 37 °C, except that Plate Count Agar was used. After incubation 72 hours on 22 °C and 48 hours 37 °C all colonies were counted. Initial plate count was enumerated in all 63 investigated bottles.
To investigate if the number of microbes will change in time after the water bottle has been opened, two bottles of each type of water (the water in first bottle was used for filtration method) was maintained at room temperatures and colony counts at 22 °C and 37 °C were analysed several times later again.

3. Results

3.1. Results of Day 1 analysis

All analysed sale items were free from *E. coli, P. aeruginosa, Enterococcus sp* and coli-form bacteria. Results of total microbial count are presented at the Table 1. No over limit levels of microbial counts were detected in flavoured waters. Several waters without additives were found to include more microbes than allowed: microbial counts in seven bottles were over the limit on both temperatures, in three bottles only on 22 ºC and in two bottles only on 37 ºC. Four mineral waters and six drinking water were over the limit on 22 °C and three mineral waters and six drinking water on 37 °C. Half of imported bottled waters (50%) and 25% local brands had total microbial count over the limits, but the quality cannot be considered to depend on the origin (p=0,203). At the same time, the biggest number of microbes was more often found in local products.

Table 1. The results of total microbial count on Day 1

	Number of bottles within limit(s) (n/%)	Number of bottles over the limit(s) (n/%)	Min and max microbial count in over limit waters (cfu/mL)
Colony Count 22 °C	53/ 84	10/16	104-1543
Colony count 37 °C	54 / 86	9 /14	104-461

3.2. Maintenance experiments

As no *E .coli*, enterococcus, coliforms nor *P. aeruginosa* were found in the first phase of study, only total microbial count was analysed after maintaining each second and third bottle of each water type on room temperature (total 42 bottles). The microbial count was detected 2 months, about 1 year and about 2 years after opening the bottles (after the first analysis). The analysis results did not show clear trends in changes of microbial counts: in most bottles the microbial count on 22 °C and 37 °C was increasing in time, but in some samples it remained similar and in some it was decreasing in time. Investigation of the bigger number of bottles of the same type is needed to make conclusions. But it was clear that issues with microbial counts were seen after maintenance only in these bottles where the microbial count was high already at Day 1. No high counts were detected in the bottles which had no, or very low number of microbes present at Day1.

4. Discussion

It is often considered that drinking water sold in bottles has higher quality than the one that is coming from tap. The study shows that especially locally produced drinking waters without additives are at risk to have high microbial count and the number of microbes might become high if maintained long time.

Even though flavoured waters include several sugars, no issues with flavoured waters were detected. In most of the cases if could be explained with addition of preservatives. People usually know that drinking flavoured waters is not too healthy due to added sugars, but the addition of preservatives is additional risk to health that is not often understood. It was noted that few bottles of flavoured waters did not contain preservatives and had a warning on the label asking the content to be used within 2 days. It looks risky if people really read the labels. Some of the flavoured waters were packed into gas environment and this also seemed to maintain the microbiological quality. The importance of gas in inhibiting bacterial growth was seen also in all carbonated waters.

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