Analysis of Selected Parameters of Bottled Water in Poland

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Abstract. *Water is essential for life. People use it in almost every aspect of their lives. Bottled water available on the market for consumption should be free from dangerous microorganisms and have a stable chemical composition.*

The presented study aimed to observe changes in selected water quality parameters in various conditions after opening a bottle of water. Four types of bottled water with different degrees of mineralization and carbon dioxide saturation were analyzed. The studies were performed according to the standards and procedures: PN-ISO 6059: 1999, PN-ISO 6332: 2001, PN-ISO 7150-1: 2002, PN-EN ISO 10523: 2012, PN-EN ISO 6222: 2004, Method 8149 – 1-(2-Pyridylazo)-2-Naphthol PAN Method.

A microbiological analysis was carried out in terms of the total number of microorganisms at a temperature of 22°C at different time intervals after opening the bottle. The analysis of statistical tests allowed for a comparison of different types of water in terms of its resistance to secondary microbiological contamination after opening the bottle, as well as for the formulation of conclusions regarding the time of storage of water in an open bottle, after which the water does not meet the quality requirements in the analyzed range.

The tested waters were also subjected to physicochemical analysis. The total content of calcium and magnesium was determined using the titration method with EDTA, ammonium nitrogen, manganese and iron using the spectrometric method, pH was measured to check the water quality and whether the tested water meets the requirements contained in the Regulation of the Minister of Health of 31 March 2011 on natural, spring waters and table waters (Journal of Laws 01.85.466). The determined physicochemical parameters of selected bottled waters were intended to characterize and determine differences in the chemical composition of these waters and to demonstrate. The obtained test results were subjected to statistical analysis, calculating the arithmetic mean, median, dispersion, standard deviation and relative standard deviation. The conducted water analysis was compared with the results obtained for tap water in Tarnow, which were made available by Tarnowskie Wodociągi Spółka z o.o.

Keywords: *microbiological analysis of water, physicochemical analysis of water, UV-VIS, bottled water, biofilm*

Introduction

Water is one of the most important chemical compounds that determines life on Earth. Its content in the human body varies in individual tissues and organs. The least water is contained in bones (22%) and adipose tissue (10%), much more is found in the liver, spleen, heart, brain and kidneys. The highest content of this component is in body secretions (plasma, lymph, sweat, bile, gastric juice). The body of an adult and healthy person contains approx. 60% water (in a newborn the water content is 75%, in the elderly approx. 50%). Water is a solvent and carrier of mineral components and is the environment for many reactions taking place in the human body, and is also necessary for the proper functioning of the digestive system. It regulates body temperature and is responsible for the transport of hormones and enzymes in the human body. Water is removed from the body through the kidneys, lungs, skin and digestive tract (Brzozowska and Gawęcki, 2008); Hoffman and Jędrzejczyk, 2004; Derkowska-Sitarz and Adamczyk-Lorenc , 2008; Flis and Konaszewski, 1986, Gajowiak and Molska, 2012). Water is obtained by the human body from various sources: from food, from consumed fluids and during the combustion of nutrients (fats, proteins, carbohydrates), (Flis and Konaszewski, 1986, Gajowiak and Molska, 2012). The demand for water depends on climate, temperature, diet and physical activity. Water must be supplied to the body continuously, because the body cannot store it. Water deficiency can lead to dehydration and serious health consequences (problems with urination, electrolyte disorders, changes in blood pressure, kidney disorders). There is a threat to life when water deficiency reaches 20%. Excess water can be equally dangerous. Single consumption of large amounts of fluids results in a risk of water excretion by the kidneys significantly exceeding the norm (Gajowiak and Molska, 2012).

In Poland, according to the Act of 25 August 2006 on food and nutrition safety, bottled water can be divided into natural mineral waters, spring waters and table waters (Brzozowska and Gawęcka, 2008).

The basic criterion for distinguishing waters and adapting them to individual needs is carbon dioxide saturation and mineral salt content (Hoffman and Jędrzejczy, 2004). Due to the degree of mineralization, in accordance with the Regulation of the Minister of Health on natural mineral waters, spring waters and table waters (Journal of Laws 2011.85.466) of March 31, 2011, bottled waters can be divided into 4 groups: very low mineralization ($\leq$50 mg of mineral salts/l of water), low mineralization (50-500 mg of mineral salts/l of water), medium mineralization (500-1500 mg of mineral salts/l of water) and high mineralization (>1500 mg of mineral salts/l of water). Bottled waters are divided into the following categories depending on their carbon dioxide saturation level: still water (0 mg CO_2 /1 water), low-saturated water ($\leq$ 1500 mg CO_2 /1 water), medium-saturated water (1500-4000 mg CO_2 /1 water) and high-saturated water (>4000 mg CO_2 /1 water).

Water intended for human consumption (including mineral, spring and table waters) is a source of minerals essential for health and life. Water from underground resources (natural mineral waters, spring waters and table waters) contains several dozen elements and chemical compounds. These include macronutrients (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Cl^{-}, SO_4^{2-}, HCO_3^{-}), secondary components (iron, nitrogen compounds, silicates, organic substances) and micronutrients (zinc, copper, iron, iodine, fluorine, manganese, cobalt, selenium and chromium). More than 90% of substances dissolved in mineral waters are the main components. Bottled water with a mineral content exceeding 15% of the daily requirement may have a health effect on the human body (Brzozowska and Gawęcki, 2008; Wojtaszek, 2006; Gratkowska-Żmuda, 2017).

Bottled water not only quenches thirst, but is a valuable source of minerals essential for the body. Water containing at least one mineral component is considered mineral water. The content of these components is precisely determined and is of great importance when considering water as natural mineral water. Some mineral waters may contain up to 70 chemical compounds and minerals, but only 10 (calcium, sodium, magnesium, carbon dioxide, bicarbonates, sulphates, fluorides, iodides, chlorides, iron) should be taken into account when selecting water (Brzozowska and Gawęcki, 2008; Wojtaszek, 2006; Gratkowska-Żmuda, 2017).

The content of individual minerals and chemical compounds in bottled water is related to the composition of rocks, hydrodynamic properties of the aquifer and geochemical processes occurring in this layer. In the case of spring waters, their composition does not have to meet strict chemical composition requirements. They are not attributed with any medicinal properties, because the mineral composition of these waters and their general properties do not differ from the composition of tap water. The role of water in the human body and life is enormous. If only because bottled water contains absorbable elements and minerals that occur in ionized form. When choosing water, the current state of health and individual needs of a person should be taken into account (Włodyka-Bergier et al., 2019; Gratkowska-Żmuda, 2019a) .

The physical, chemical and organoleptic properties of water are determined by its chemical composition (Macioszczyk and Dobrzyński, 2002; Bodora, 2016). The most important physical properties include: temperature, electrical conductivity, redox potential and organoleptic properties (transparency, colour, smell, taste, turbidity). Chemical properties include: pH, total nitrogen, ammonium nitrogen, nitrate nitrogen, nitrite nitrogen, chemical oxygen demand (COD), chlorides, carbon dioxide, acidity, manganese, hardness, alkalinity, iron (Hermanowicz et al., 1999).

Water can pose a serious health risk if it does not meet the relevant requirements contained in the Regulation of the Minister of Health of 7 December 2017 on the quality of water intended for human consumption (Journal of Laws 2017, item 2294). Water can be a source of contamination and transfer of microorganisms, which is why water quality monitoring is so important. It consists in checking the quality of water and provides information on whether the tested water meets the guidelines contained in the regulation on water intended for human consumption (Journal of Laws 2017, item 2294) (Gratkowska-Żmuda, 2019b).

The Regulation of the Minister of Health on natural mineral waters, spring waters and table waters (Journal of Laws 2011.85.466) of 31 March 2011 does not contain any specific requirements for bottled waters concerning maximum levels of elements and chemical compounds (exception: manganese - <0.50 mg/l), as well as pH and water hardness. These parameters are very different for each water and depend on the content of individual mineral components. This regulation very precisely specifies the requirements related to microbiological analysis.

Water intended for human consumption should not come into contact with organic substances that are a potential source of nutrients for microorganisms, which in turn may contribute to the formation of biofilm (Szczotko, 2008).

Bacteria and fungi that arise in the natural environment tend to adsorb at the solid-liquid, gas-liquid and liquid-liquid interfaces. Microorganisms that tend to form aggregated micro- and macrocolonies are called biofilms, which have the properties of attaching to the substrate (Cłapa et al., 2016; Kołwzan, 2011).

Biofilm is characterized by significant growth, which allows microorganisms to survive unfavorable conditions and use any niche. A mature biofilm is a three-dimensional, compact structure that can consist of one or many species of microorganisms and can be single- or multi-layered. Between the biofilm structures there are networks of channels filled with fluid. They connect the environment with the interior of the biofilm. The cells that make up the biofilm are limited by an extracellular polymeric substance - EPS (extracellular polymeric substances). The components of EPS play an important role in the construction and functioning of the biofilm. EPS constitutes 90% of the entire mass of the mature biofilm, and the remaining 10% are microorganisms. EPS consists of: water, DNA, surfactants, polysaccharides, nucleic acids, proteins and phospholipids. The structure of the extracellular polymers of the biofilm depends on the species of bacteria. The qualitative and quantitative composition of the supplied nutrients affects the amount of polymer produced. The factors influencing its production are: reduced amounts of nitrogen, potassium and phosphates, pH of the medium and the incubation temperature. Biofilm bacterial cells have different functions and properties than cells living in a free form. Microorganisms living in larger clusters provide themselves with protection against the adverse effects of external factors and create an environment of easier access to nutrients. Biofilm formation is a multi-stage process dependent on the properties of the colonized surfaces and structure, as well as on the properties of the microorganisms. Intercellular (Cłapa et al., 2016; Kołwzan, 2011; Cybulska, 2018; Czyżerwska-Dors et al., 2018; Szczotko, 2019).

Biofilm formation is a significant problem for the drinking water industry as a potential source of bacterial contamination, including pathogens, and in many cases also affects the taste and odor of drinking water and promotes pipe corrosion. Biological contamination of water is largely caused by microbiological growths forming on the surface of the water supply system. The formation of biofilm in tanks and in the water supply network poses a serious threat to consumers. The materials from which the water supply network is constructed affect the quality of water. It has been confirmed that flexible pipes, coatings, foils and sealing materials are conducive to the growth of microorganisms. Biofilm develops favorably on materials made of steel. However, copper pipes become less favorable for biofilm. Materials with which water comes into contact should not deteriorate the microbiological quality of water. In order to improve water quality, disinfection should be used and materials that could negatively affect the quality of water intended for human consumption should be eliminated. Studies have shown that temperature fluctuations potentially affect not only the initial attachment of bacteria to surfaces, but also the rate of biofilm growth. Microbiological growths cause dangerous threats related to the proper water quality, as well as decide about the change of the physicochemical composition in the water supply network, (Liu et al., 2016; Świderska-Bróż, 2012; Szczotko, 2007).

Materials and Methods

The tests of bottled water available in stores were conducted in the summer. The waters were purchased in the city of Tarnów and tested in the Water and Sewage Testing Laboratory of Tarnowskie Wodociągi Sp. z o.o. Four types of water were analyzed: carbonated (G) and non-carbonated (NG), each in a variant with low (NM) and high (WM) mineral content. The individual packages of each type of water came from the same batch.

The first water tested was natural mineral water extracted in Beskid Sądecki (1/NG/WM). This water is low-saturated with carbon dioxide (up to 1500 mg/l CO_2 concentration) and highly mineralized. The water was in 1.5-litre PET bottles. This water contained about 1700 mg/l of dissolved minerals. Anions constitute 77% by weight of minerals in water (1/NG/WM), while cations constitute 23%. Among the anions, the bicarbonate ion predominates – HCO_3^- about – 1270 mg/l, and among the cations, the calcium ion Ca^{2+} predominates – about 170 mg/l.

The second type of water used for analysis was spring water obtained in the Częstochowa region (2/G/NM). This water is a low-mineralized water with high carbon dioxide saturation. According to the manufacturer, the water should be consumed within 48 hours of opening, stored in a cool and dark place and protected from freezing. Table 2 presents the content of individual mineral components. The water was in 1.5-liter PET bottles. Water extracted in the Częstochowa region (2/G/NM) consists of 408.2 mg/l of minerals. Hydrogen carbonate ions constitute 76% of the weight of mineral components in water. Cations constitute the remaining 24%. Among the cations, calcium ions play the dominant role.

The next tested and analyzed water was a popular mineral water extracted in the north-eastern part of the Silesian Voivodeship (3/NG/NM). This water is a low-mineralized, non-carbonated and low-sodium water. The label contains information that the water is suitable for people on a low-sodium diet. It has a positive opinion from the Institute of Mother and Child in the nutrition of children over 1 year of age. The manufacturer recommends drinking the water within 24 hours of opening and protecting it from sunlight and storing it in a cool, dry place. The water was in 1.5-liter PET bottles. This water contains about 500 mg/l of dissolved minerals. Water (3/NG/NM) contains anions constituting 78% by weight of minerals in water, of which the bicarbonate ion constitutes as much as 68% and 22% of cations, of which 13% are calcium ions.

The last water tested was natural mineral water also extracted in Beskid Sądecki (4/G/WM). This water is highly saturated with carbon dioxide (containing over 4000 mg/l CO_2) and highly mineralized. It contains significant amounts of calcium and magnesium. The manufacturer included information on the label regarding the storage conditions of the water - in a cool and dark place at a temperature of 4°C to 20°C. The label did not include guidelines regarding the time after opening the water is suitable for drinking. Water 4/G/WM contains 2100 mg/l of minerals. Anions constitute 75% of the weight of mineral components in water, of which bicarbonate ions are as much as 74%. Cations constitute 23%, and the dominant cation is calcium ion, constituting 15% (300 mg/l).

The water bottles were opened at the beginning of the experiment and then the water was stored in different conditions simulating the usual storage of this product at home: on a table, near a window (sunny place, ambient temperature) and in a refrigerator (dark place, refrigeration conditions).

Each of the waters was subjected to microbiological analysis in terms of the total number of microorganisms at a temperature of 22°C and physicochemical analysis using several research methods and procedures: pH, total calcium and magnesium content, iron, manganese and ammonium nitrogen were determined.

The determination of the total content of calcium and magnesium involved complexometric titration of calcium and magnesium contained in the tested water with a disodium versatate (EDTA) solution in an alkaline environment using mordant black 11 as an indicator, PN-ISO 6059: 1999.

Ammonium nitrogen in water samples was determined by spectrophotometric method. The determination consisted in the reaction of ammonium nitrogen with salicylate and chlorate(I) ions in the presence of sodium pentacyanonitrosylferrate(III), PN-ISO 7150-1: 2002.

In water tests , a method was used to determine low concentrations of manganese. Ascorbic acid was used to reduce the oxidized form of manganese to Mn^{2+} . A solution of 1-(2-Pyridylazo)-2-naphthol (PAN) combined with Mn^{2+} ions, forming an orange-colored complex, Method 8149 – 1-(2-Pyridylazo)-2-Naphthol PAN Method.

Iron was determined by spectrometric method using 1,10-phenanthroline. The determination consisted in reducing iron(III) to iron(II) using hydroxylamine in acidic medium PN-ISO 6332: 2001.

The total number of microorganisms was determined for all water samples. Water samples were placed on nutrient agar and incubated at 22°C. For incubation, the plates with the tested water samples were stored in an incubator under aerobic conditions at 22°C PN-EN ISO 6222: 2004.

The obtained test results for water were compared with the results for tap water, as the basic source of drinking water supply to residents.

Results and Discussion

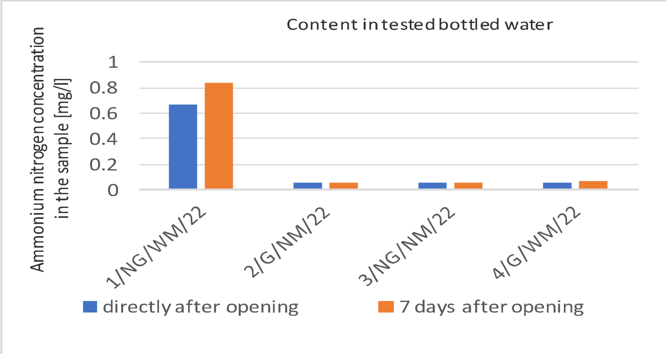
The test results for different types of bottled water were compared with the results of tests for water intended for human consumption, i.e. tap water (Table 1).

Table 1 Results of selected parameters within the basic scope of tests of drinking water supplied to the water supply network on 15/07/2019.

No.	Tested parameter	Unit	The value of the tested tap water from the Zbylitowska Góra Water Treatment Plant	Permissible value
Bacteriological index of water quality				
1.	Total number of microorganisms at 22°C after 72h	jkt/1 ml	0	100
Physicochemical indicators of water quality				
2.	**pH**	-	7.3	6.5-9.5
3.	**Ammonium ion**	mg/l	<0.052	0.50
4.	**Manganese**	μg /l	16	50
5.	**Iron**	μg /l	11	200
6.	**Hardness**	mg $CaCO_3$ /l	150	60-500

For tap water, the requirements are clearly specified in the Regulation of the Minister of Health of 7 December 2017 on the quality of water intended for human consumption (Journal of Laws 2017, item 2294). Of the several dozen chemical and physical quality parameters for which parametric values have been specified, the pH of tap water should be in the range of 6.5 to 9.5. The permissible iron content in tap water is 200 μg/l, and manganese – 50 μg/l. In turn, the parametric value of ammonium ~~ion~~ in water given to people for drinking is 0.50 mg/l. As regards microbiological parameters, in addition to the key indicators of microbiological water contamination, such as coliform bacteria, Escherichia coli or fecal enterococci, tap water is also tested for the total number of microorganisms at a temperature of 22°C and *Pseudomonas aeruginosa,* parameters that are mandatory for bottled water.

Figure 1. Ammonium nitrogen content in bottled water



The graph in Figure 1 shows the ammonium nitrogen content for each of the tested waters immediately after opening the package and 7 days after opening it. The highest ammonium nitrogen content (0.665 mg/l) immediately after opening the package was found in water no. 1/NG/NM/22 (non-carbonated, highly mineralized). In the remaining waters, the ammonium ion concentration was below the detection limit (<0.052 mg/l). An increase in the ammonium nitrogen concentration was observed after 7 days only in the case of water sample no. 1/NG/NM/22 (0.834 mg/l).

Figure 2 pH of bottled water

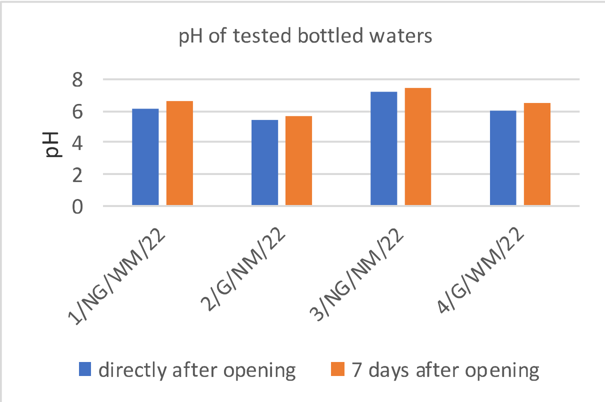


Figure 2 shows graphs of changes in pH values of the tested water samples depending on the time at which the pH measurement was made after opening the package. An increase in pH of the tested waters was observed 7 days after opening the package.

Figure 3 Total calcium and magnesium content in the tested water samples immediately after opening the package

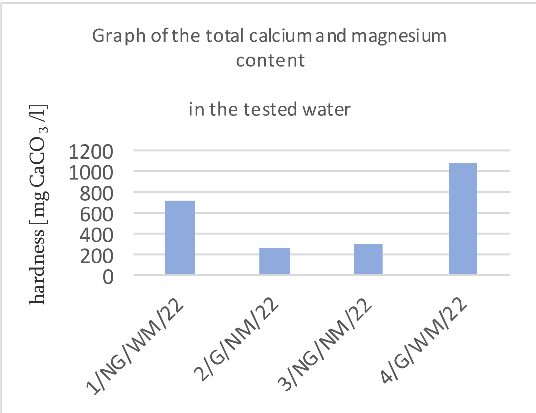


Figure 3 presents a summary of the total calcium and magnesium content in individual waters. The waters were tested for the total calcium and magnesium content immediately after opening. The hardest water was 4/G/WM/22 containing 1091 mg $CaCO_3$ mg/l and 1/NG/WM/22 containing 733 mg $CaCO_3$ mg/l. The remaining waters 2/G/NM/22 (265 mg $CaCO_3$ mg/l) and 3/NG/NM/22 (314 mg $CaCO_3$ mg/l) belong to low-mineralized waters.

Figure 4. Content of manganese and iron in the tested water samples immediately after opening the package

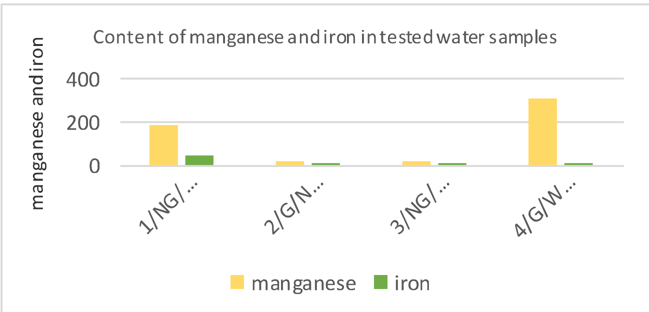
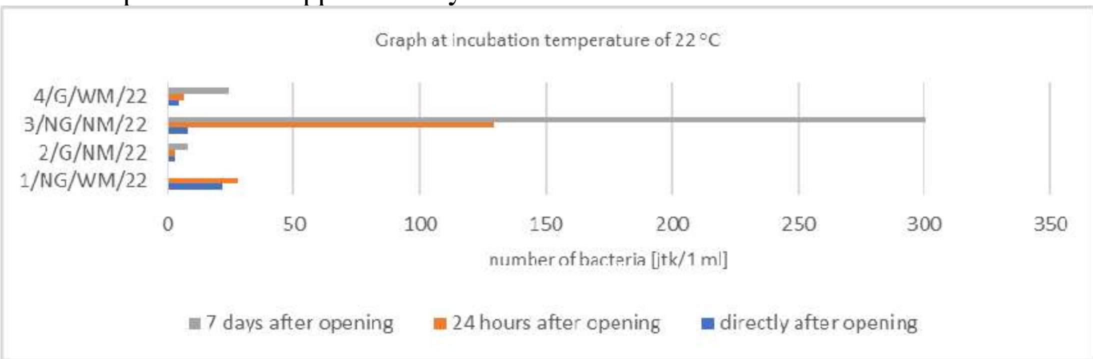


Figure 4 presents a comparison of manganese and iron content determined for the tested water samples immediately after opening. The highest concentration of manganese was observed in sample 4/G/WM/22 (313 µg/l). In sample 1/NG/WM/22 the amount of manganese was 186 µg/l. In the remaining samples 2/G/NM/22, 3/NG/NM/22 the manganese concentrations were 15 µg/l and 18 µg/l, respectively. The highest concentration of iron was in sample 1/NG/WM/22 and amounted to 43 µg/l. In the remaining samples 2/G/NM/22, 3/NG/NM/22 and 4/G/WM/22 the iron content was 10 µg/l, 10 µg/l and 13 µg/l, respectively.

The tested bottled waters, which were stored in refrigerated conditions and in a sunny place, were subjected to microbiological analysis for the total number of microorganisms at a temperature of 22°C. The graph below (Figure 5) shows the number of colonies in 1 ml of bottled water stored at a temperature of approx. 20°C.

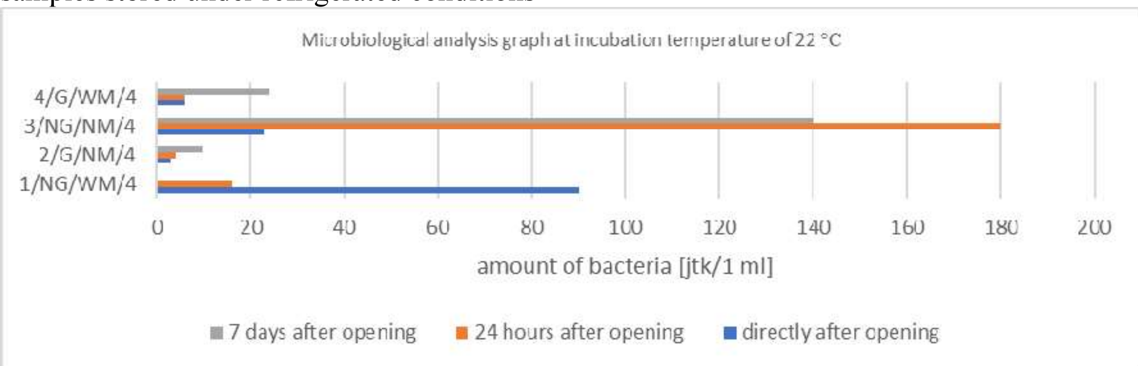
Figure 5. Microbiological analysis graph at an incubation temperature of 22°C for bottled water samples stored at approximately 20°C



When analyzing the graph showing the total number of microorganisms in the tested water samples at a temperature of 22°C, it was noted that immediately after opening, after 24 hours and after 7 days from opening, there were colonies of microorganisms in each water. In the case of water no. 3/NG/NM/22, a small number of microorganisms was determined immediately after opening (8 jtk/1ml). After 24 hours, a significant increase in bacteria was observed (130 jtk/1ml), and after 7 days the number of colonies was 300 jtk/1ml. In the remaining waters, the number of bacteria in all time intervals does not exceed 40 jtk/1ml.

Figure 6 presents a summary of the number of colonies in 1 ml of each bottled water stored at a temperature of approximately 4°C.

Figure 6. Microbiological analysis graph at 22°C incubation temperature for bottled water samples stored under refrigerated conditions



Analyzing the graph in Figure 6 on the total number of microorganisms in the tested water samples at a temperature of 22°C, it was noticed that immediately after opening, after 24 hours and after 7 days from opening, there were colonies of microorganisms in each water. In the case of water no. 3/NG/NM/4, after 24 hours at a temperature of 22°C, colonies of microorganisms were observed (180 jtk/1ml), and after 7 days the number of colonies was 140 jtk/1ml. Many colonies of bacteria were observed in water 1/NG/WM/4 immediately after opening the package, which contained 90 jtk/1 ml. After 24 hours from opening, the number of bacteria decreased (16 jtk/1ml), and after 7 days the water did not contain any microorganisms. In the remaining waters, the number of bacteria in all time intervals did not exceed 25 jtk/1ml.

High microbial growth was observed in still, low-mineralized water (3/NG/NM) stored both at about 20°C and at about 4°C. The waters came from the same batches, which would mean that the entire batch would have a similar total number of microorganisms at 22°C.

Conclusions

Bottled waters were tested to check their quality and to show differences in the chemical composition of various bottled waters. Mineral and spring waters were also subjected to microbiological analysis to determine the total number of bacteria at a temperature of 22°C and how this parameter changes as a function of time and temperature of water storage.

The study has shown that the content of ammonium nitrogen in sample number 1/NG/WM/22, stored at a temperature of about 20°C, increases after 7 days from opening the bottle, which could be caused by an increase in pH and temperature. The "original" state of water is also disturbed by factors such as temperature, pressure and, above all, contact of water with oxygen contained in the air.

The study has confirmed that waters containing carbon dioxide are acidic. Waters containing high levels of carbonates and bicarbonates, alkali metals are alkaline. The pH of bottled waters ranges from 5.5 to 7.20. The increase in pH after 7 days from opening the bottle may be largely due to the loss of carbon dioxide in the case of carbonated waters, and in the case of waters with a low carbon dioxide content, probably due to hydrolysis reactions of anions originating from weak acids. The change in pH may also be influenced by changes in temperature and the place where the samples are stored.

It has also been confirmed that 4/G/WM/22 water contains a large amount of calcium and magnesium, which is consistent with the information on the label. Each bottled water has a different overall hardness, which is caused by different concentrations of calcium and magnesium.

None of the tested waters exceeded the permissible manganese content specified in the Regulation of the Minister of Health (Journal of Laws 01.85.466) (maximum manganese concentration - 0.50 mg/l). The highest iron concentration was recorded in sample 1/NG/WM/22 and amounted to 43 μg/l.

The study has shown that carbonated waters are probably more resistant to secondary contamination. The producer of water no. 3/NG/NM recommends consuming it within 24 hours of opening. Studies have confirmed this information. After just 24 hours of opening the package, the number of colonies increased above the permissible standard. In the remaining waters (1/NG/WM, 2/G/NM, 4/G/WM) after 24 hours, and even after 7 days of opening, the content of bacteria did not exceed the permitted amount. Based on the studies conducted, it is not possible to state that the tested waters 1/NG/WM/, 2/G/NM, 4/G/WM can be consumed even after seven days of opening the package, so it can be stated that the values of the parameters tested in waters after 7 days of opening do not constitute a contraindication to consuming the aforementioned waters.

There was no effect of the storage temperature of bottled water after opening on the degree of growth of bacterial colonies in them. Their multiplication was the same for a given water at room temperature and in refrigeration conditions.

Comparing the pH of tap water with the tested mineral waters, it was observed that one of the tested bottled waters (3/NG/NM) has a pH similar to water supplied by the water supply network. The remaining waters have a lower pH than tap water. The hardness of tap water cannot exceed 500 mg $CaCO_3$ mg/l. However, for bottled waters, there is no specific range in which bottled waters should be. Mineral waters may contain more calcium and magnesium ions because these waters are not used as the main source of drinking water supply.

The probability of microorganisms developing in bottled water is greater than in tap water. Water from the tap network is very often monitored and is constantly available in the water network. Bottled water is monitored before leaving the warehouse and distribution. The manufacturer determines the type and frequency of such monitoring in the HACCP system. Water that is monitored sporadically may contain microorganisms. If the total number of bacteria is determined before introducing the mineral water into circulation and it is significant, then this batch of water is withdrawn. Tap water provides numerous mineral components that are essential in the daily diet. The appropriate mineral content is provided by highly mineralized waters.

The studies show that 4/G/WM water could be consumed after a longer period of time after opening. Probably the main reason for the low bacterial growth in this water was its high carbon dioxide saturation. The studies show that 3/NG/NM water should not be consumed even after 24 hours of opening. The bacterial growth observed in this water was the highest among the four waters tested. Highly mineralized waters can be a source of minerals essential in the diet. There are different opinions on which waters are more beneficial to health: carbonated or still. This study has shown that carbonated waters are more resistant to the growth of microorganisms.

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