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Research Article

The effect of the reverse osmosis bottled water storage on its bacteriological, chemical and physical properties

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Abstract: This study aimed to study the effect of the reverse osmosis bottled water storage on its bacteriological, chemical and physical properties. After storing the bottled water for 90 days, the number of bacteria decreased from 49 to 9 CFU/1ml for aerobic bacteria and 3.6-1.1 MPN/100ml for the total number of total coliform. Fecal coliform and *Escherichia coli* for laboratory W1 ranged 7-16CFU/1ml for the total number of aerobic bacteria and from 1.1-2.2 MPN/100ml for *Pseudomonas aeruginosa* for the W3 laboratory. After 90 days of storage, an increase in temperature (20.8-43°C), decrease in pH (5-5.4), a high conductivity (125.1-119µS/cm), a rise in dissolved solid salts (77.4-81.2) and total hardness (24.2_29.1) were recorded. Calcium (9.8-12.7), magnesium (3.5-6.5), chlorides (14.7-18.1), sulfate (3.3-5.0), sodium (8.7-12.3), potassium (0.1-0.4) and high nitrate (0.6-3.3) mg/l also were recorded. The bacterial numbers increased during storage process and the storage process also affected all the physical and chemical properties of the bottled water bottles. The results showed significant differences between the studied laboratories as well as between the months at the level. It also showed significant differences between the bacterial and physicochemical treats after 30, 60 and 90 days.

Keywords: Reverse Osmosis, Bottled water, Escherichia coli, Fecal coliform.

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Introduction

Water is essential for life and a favorable supply must be accessible to people for drinking and health. Every exertion ought to be made to realize a drinking-water quality as secure as practicable (World Health Organization 2006). There is almost a global shortage of water and ranked as the world's most urgent need (Abdudu et al. 2013). The provision of clean and safe drinking water is one of the major infrastructural problems in growing countries and that is because the majority of people do not have access to reliable potable water sources.

The quest for cheap and readily available sources of potable water has led to the emergence of sachet water. Packaged water is defined as any potable water processed and offered for sale in sealed food-grade bottles or other appropriate containers for human consumption (Ojekunle & Adeleke 2017). Bottled water can be defined as any potable water that is manufactured distributed, or offered for sale, which is sealed in food-grade bottles or other sanitary containers and intended for human consumption (FDA 1995). Bottled water is the fastest-growing drink choice and its consumption in the world increased by an average of 10%, therefore it becomes the most dynamic sector of all food and beverage industries due to accessibility, relatively low cost, better taste, and lower level of impurities (EPA) (EPA 2003). The main reason for this rapid consumption was the lack of safe and accessible drinking water and the taste of chemicals, particularly chlorine, used to purify tap water (Samadi et al. 2009). Bottled water is a beverage that is rapidly gaining in popularity World consumption in 2007 was in excess of 200 million liters (World's Water 2010). The average annual consumption is

Table 1. The results of bacterial tests for bottled water for the laboratories under study, which showed positive results in different storage periods.

Factory	Storage periods	Storage periods (day)			
W1		90	60	30	0
	A.P.C. numbers. per ml	9	56	76	49
	T.C. numbers. per 100ml	1.1	5.1	6.9	3.6
	F.C. numbers. per 100ml	1.1	5.1	6.9	3.6
	E.coli numbers per 100ml	1.1	5.1	6.9	3.6
		L.S.D. at the level of significance 0.05 =1.509			
W3	A.P.C. numbers. per ml	16	41	24	7
	Numbers of *P. aeruginosa* per 100 ml	2.2	1.1	1.1	1.1
		L.S.D. at the level of significance 0.05 =1.509			

L.S.D = at the level of significance ≤0.05

over 100 liters per person in 15 countries from the world ranking, and over 200 liters per person in the countries at the top of this ranking, namely Mexico, Italy, and the United Arab Emirates (Malwina et al. 2013). Water is one medium through which pathogenic organisms are spread. Sachet or packaged water, which is the major means of drinking water, is not exempted from deadly organisms, due to its microbial substance, unsafe water supply, and unhygienic environment are fingered as the cause of over 70% of diseases in developing countries (Adekunle et al. 2004). Woods (2010) reported that there was a significant deterioration in the microbiological quality of the most of the tested sachet water samples when stored at temperatures higher than refrigeration temperature. Adequate consumption of clean, safe, and potable water is a basic requirement for humans to maintain good health and balance their body fluids (Oluwafemi et al. 2021). Pathogens could be introduced into sachet water in several ways, which include poor production and packaging, poor treatment, and inappropriate handling and storage. The quest by sachet water production enterprises to be on top of the competition and regularly meet their customer's demands has led to the production of large quantities of sachet water which is stored in their respective factories awaiting purchase. During storage of the sachet water at the factory or by the respective retail customers in their stores, or at the consumer home, pathogens could be introduced into the sachet water depending on how long and under what hygienic condition the water is stored (Ohwo 2018). Safety concerns in stored drinking water include pathogenic microorganisms and other contaminants. The presence of coliform bacteria in water can signify poor sanitation and the possibility of pathogenic bacteria (Stephanie et al. 2015). The test for total coliforms is often performed simultaneously with *Escherichia coli*. Although most *E. coli* strains are harmless, a few strains are pathogenic, hence, the presence of *E. coli* would be of particular concern (Edberg et al. 2000). Hence, this study aimed to study the effect of the reverse osmosis bottled water storage on its bacteriological, chemical and physical properties.

Materials and methods

The samples were collected bi-monthly, starting from the second half of October 2020 until the first half of April 2021, and six brands that are considered the most consumed and traded in the holy city of Karbala and some governorates were selected. Samples were collected before treatment (liquefied water) and after treatment with reverse osmosis (bottled water) to perform bacterial, physical and chemical tests.

Analysis of the total number of aerobic bacteria was done using the Pour pate method (APHA 1985). Measuring of total coliform bacteria, fecal coliform,

Table 2. Temperature in different storage period.

Sample	Temperature 0 day storage	Temperature 30 day storage	Temperature 60 day storage	Temperature 90 day storage	Average
W1	20.9	34.9	37.9	42.4	34.03
W2	20.8	35	38.4	44	34.55
W3	20.7	35	38.1	43.7	34.38
W4	20.7	35.2	38.3	43	34.30
W5	20.9	35.2	37.8	43.6	34.38
W6	20.7	35.8	38.5	44.2	34.80
L.S.D.		0.736			0.368
average	20.8	35.2	38.2	43.5	
L.S.D.		0.301			

E. coli and *Pseudomonas aeruginosa* were performed according to (APHA 2017).

Temperature, pH, electrical conductivity, and TDS were measured using multi meter instrument (HI 9633, HANNA) (APHA 2017). Total hardness, calcium, magnesium, potassium, sodium, chloride and nitrate were measured according to (APHA 2017) and sulphate based on (APHA 2003).

Results and Discussion

The bacteriological properties were studied after storing the six bottled water samples for a period of 90 days, and the results were recorded (Table 1) that show increasing in the number of aerobic APC bacteria in the W1 laboratory from 49CFU/ml at the beginning of the storage period to 76CFU/ml.

At the storage period of 30 days, the growth results decreased sharply at the end of the storage period (90 days) to 9CFU/ml, while the APC of the W3 laboratory increased from 7CFU/ml at the beginning of the storage period to 24 CFU/ml at the storage period of 30 days and the growth results decreased at the end of the final storage period i.e. 90 days to 16CFU/ml.

Regarding the results of T.C, FC and *E. coli* for W1 laboratory, they were recorded at the beginning of the storage period as 3.6 MPN/100ml and then increased to 6.9 MPN/100ml and the results of bacterial growth decreased sharply at the end of the final storage period to 1.1 MPN/100ml, while the storage results for B increased at the storage period of 30 days to 6.9 MPN/100ml and decreased at the end of the storage period to 1.1 MPN/100 ml. In the study conducted by (17), the presence of total colon, fecal colon and *E. coli* bacteria was recorded in 50% of the water samples that were examined during a period before storage, while the decrease or disappearance of bacterial numbers was recorded in some samples during the period of 8 and 12 weeks of storage. As shown in Table 1, the W3 model recorded a remarkable growth of *Pseudomonas aeruginosa* bacteria at the end of the storage period that was 90 days, 2.2MPN/100. The presence of the bacteria in the water indicates poor water disinfection or the occurrence of contamination inside the water filling plants. The heterotrophic bacteria increase logarithmically during storage periods and the death and autolysis of the natural flora provides food and support for heterotrophic bacterial, making them multiply at high levels the storage period (Shahryari et al. 2020) at a significance level of as seen in the present study ($P \leq 0.05$) (Table 1).

The temperature during storage period was 20.7, while in during 30-day storage was 35.1 and at the 60 days it rose to 38.1, and at the 90 days it was recorded 43.4. These results are in line with report of (Isikwue & Chikezie 2014). The results showed a significant difference in the temperature at the 90-day storage period with the other storage days ($P \geq 0.05$) (Table 2). The pH of water was 5.3 during storage period, while in the storage period of 30 days it was 5.2, at the 60, 5.1, and at 90 days, 5 (Table 3). These results

Table 3. pH in different storage periods.

Sample	pH day storage 0	pH 30 day storage	pH 60 day storage	pH 90 day storage	Average
W1	5.1	5	4.9	4.7	4.9
W2	5	4.9	4.8	4.8	4.9
W3	5.1	5	5	4.9	5.0
W4	5.5	5.4	5.2	5	5.3
W5	5.3	5.2	5.1	5	5.2
W6	6.2	5.8	5.7	5.8	5.9
L.S.D.		0.692			0.346
average	5.4	5.2	5.1	5.0	
L.S.D.		0.283			

Table 4. Electrical conductivity in different storage periods.

Sample	E.C day storage 0	E.C 30 day storage	E.C day storage 60	E.C 90 day storage	Average
W1	98.2	100.6	103.4	101	100.80
W2	91.3	92.1	95.7	98.6	94.43
W3	93.8	94.5	97.3	96.7	95.58
W4	182.9	185.6	188.3	191.1	186.98
W5	91.9	93.4	96	99.5	95.20
W6	155.7	155.8	159.2	163.6	158.58
L.S.D.		74.299			37.149
average	119.0	120.3	123.3	125.1	
L.S.D.		30.332			

Table 5. The dissolved solid salts in the different storage.

Sample	Dissolved solid salts 0 day storage	Dissolved solid salts 30 day storage	Dissolved solid salts 60 day storage	Dissolved solid salts 90 day storage	Average
W1	64.00	65.39	67.21	65.65	65.56
W2	59.00	59.87	62.21	64.09	61.29
W3	61.00	61.43	63.25	62.21	61.97
W4	119.00	120.64	122.40	124.22	121.56
W5	60.00	60.71	62.40	64.68	61.95
W6	101.20	101.27	103.48	106.41	103.09
L.S.D.		48.36			24.178
average	77.4	78.2	80.2	81.2	
L.S.D.		19.741			

were similar to the findings of (Hassan et al. 2020; Daffi & Wamyil 2021), which showed temperature effects pH with an increase storage time and exposure to sunlight. The results showed that there were significant differences in the pH at the storage period of 0 days with those of 60 and 90 days ($P \leq 0.05$).

The electrical conductivity in 0 day was 118.9µS/cm reached in the 30-day to 120.3µS/cm, at the 60 days to 123.3µS/cm and at the 90 days to 125.0µS/cm (Table 4). The results of the study are similar to Muhamad et al. (2011), who studied the effect of storage temperature and exposure to sunlight on the physical and chemical properties of the bottled water in the Kurdistan region of Iraq and showing a high conductivity values after exposure to the sun due to leaching of ions and minerals from plastic containers. Therefore, the EC values increase

Table 6. The total hardness in the different storage.

Sample	Hardness 0 day storage	Hardness 30 day storage	Hardness 60 day storage	Hardness 90 day storage	Average
W1	23.53	24.50	25.70	27.10	25.21
W2	21.57	22.60	24.20	26.30	23.67
W3	21.57	22.00	25.00	27.70	24.07
W4	21.57	21.80	23.40	24.00	22.69
W5	19.61	21.70	24.30	26.70	23.08
W6	37.46	38.50	40.10	43.00	39.76
L.S.D.		12.18			6.088
average	24.2	25.2	27.1	29.1	
L.S.D.		4.971			

Table 7. Calcium in different storage periods.

Sample	Calcium day torage 0	Calcium 30 day storage	Calcium 60 day storage	Calcium 90 day storage	Average
W1	10.20	10.80	11.50	13.10	11.40
W2	10.20	11.30	11.90	12.80	11.55
W3	8.10	9.00	9.20	11.40	9.43
W4	10.20	10.70	11.80	13.70	11.60
W5	8.10	9.60	10.70	12.00	10.10
W6	12.20	11.00	11.90	13.00	12.03
L.S.D.		2.06			1.029
average	9.8	10.4	11.2	12.7	
L.S.D.		0.840			

Table 8. Magnesium in different storage periods.

Sample	Magnesium 0 day storage	Magnesium 30 day storage	Magnesium 60 day storage	Magnesium 90 day storage	Average
W1	3.20	4.10	5.00	5.80	4.53
W2	2.80	4.00	4.90	5.40	4.28
W3	3.30	3.90	4.70	5.90	4.45
W4	2.80	3.30	5.30	6.20	4.40
W5	2.80	3.80	4.60	5.60	4.20
W6	6.30	7.10	8.50	10.50	8.10
L.S.D.		2.87			1.434
average	3.53	4.37	5.50	6.57	
L.S.D.		1.171			

with the increase in the filtration of ions by the effect of sunlight. However, the results o did not show significant differences in the electrical conductivity at the different storage periods. The TDS was recorded at storage period of 0 days as 77.3mg/liter, for 30 days as 78.2mg/liter, and at the 60 days as 80.1mg/liter and at the 90 days as 81.2mg/L (Table 5). The results did not show significant differences in the dissolved solid salts at the different storage stages.

The total hardness was recorded at the 0-day storage period as 24.2mg/l, in 30-day storage as 25.1mg/l, at the 60 day, 27.1mg/l, and 90-day as 29.1mg/l (Table 6). Ajekunle et al. (2015) recorded an increase in the total hardness in the bottled water, where the average hardness values increased from 25.67±5.57 and 27.90±6.05mg/l, respectively to 33.77±3.51 and 36.85±4.79mg/L after three months of storage. The results showed significant differences in the total hardness at the 90-day storage period

Table 9. sulfates in different storage periods.

Sample	Sulfates 0 day storage	Sulfates 30 day storage	Sulfates 60 day storage	Sulfates 90 day storage	Average
W1	4.60	4.60	4.60	4.60	4.60
W2	1.22	1.00	0.80	0.60	0.91
W3	5.78	5.10	5.00	3.70	4.90
W4	6.10	5.40	4.80	3.60	4.98
W5	5.20	4.50	3.60	3.10	4.10
W6	7.20	6.50	5.50	4.20	5.85
L.s.d.		3.26			1.632
average	5.02	4.52	4.05	3.30	
L.s.d.		1.333			

Table 10. Chlorides in different storage periods.

Sample	Chlorides 0 day storage	Chlorides 30 day storage	Chlorides 60 day storage	Chlorides 90 day storage	Average
W1	15.60	16.80	18.00	18.80	17.30
W2	13.80	14.50	15.70	17.10	15.28
W3	11.90	12.70	13.90	15.00	13.38
W4	12.10	13.30	14.40	15.70	13.88
W5	10.60	11.60	12.40	14.30	12.23
W6	24.50	24.90	25.50	27.80	25.68
L.S.D.		9.04			4.519
average	14.75	15.63	16.65	18.12	
L.S.D.		3.690			

Table 11. Potassium in different storage periods.

Sample	Potassium 0 day storage	Potassium day storage 30	Potassium 60 day storage	Potassium 90 day storage	Average
W1	0.30	0.20	0.10	0.00	0.15
W2	0.40	0.33	0.30	0.10	0.28
W3	0.40	0.40	0.10	0.00	0.23
W4	0.50	0.45	0.30	0.10	0.34
W5	0.30	0.23	0.00	0.00	0.13
W6	0.80	0.42	0.20	0.40	0.46
L.S.D.		0.27			0.133
average	0.45	0.34	0.17	0.10	
L.S.D.		0.109			

($P \geq 0.05$). Calcium levels were recorded at the 0-day storage period as 9.8mg/L, in the 30-day storage as 10.4mg/L, at the 60 days as 11.2mg/L and at the 90 days as 12.7mg/l (Table 7).

Akharame et al. (2018) studied the effect of sunlight on the quality of bottled water and found high calcium values of 0.60±2.85, 0.64±4.49 and0.96±8.34 at 0, 14and 28 storage days, respectively. The results showed significant differences between calcium levels at the storage stages ($P<0.05$). Magnesium levels were recorded at 0 days as 3.4mg/L, at the 30-day as 4.2mg/L, at the 60 days as 4.8mg/L, and at the 90 days as 5.6mg/l (Table 8). The results were in line with the findings of, where the Mg values were 0.20±3.70, 0.59±4.12 and 0.20±4.87 in 0, 14 and 28 days of storage, respectively. The increase in Ca and Mg is the result of an increase in total hardness. The results showed significant differences between Mg at the 90-day storage phase with 0 and 30 days ($P<0.05$).

Table 12. Sodium in different storage periods.

Sample	Sodium 0 day storage	Sodium 30 day storage	Sodium 60 day storage	Sodium 90 day storage	Average
W1	7.80	7.10	6.60	4.90	6.60
W2	12.10	11.00	9.70	8.60	10.35
W3	9.40	8.90	7.90	6.30	8.13
W4	18.50	16.20	14.10	11.90	15.18
W5	7.90	7.10	5.90	4.70	6.40
W6	18.30	18.20	16.30	15.80	17.15
L.S.D.		8.42			4.212
average	12.33	11.42	10.08	8.70	
L.S.D.		3.439			

Table 13. Nitrates in different storage periods.

Sample	Potassium 0 day storage	Potassium 30 day storage	Potassium 60 day storage	Potassium 90 day storage	Average
W1	0.84	1.90	2.50	3.40	2.16
W2	0.59	1.40	2.30	3.60	1.97
W3	0.67	1.20	2.10	2.90	1.72
W4	0.40	1.30	2.50	3.90	2.03
W5	0.64	1.90	2.80	3.70	2.26
W6	0.60	1.10	1.90	2.80	1.60
L.S.D.		0.61			0.306
average	0.62	1.47	2.35	3.38	
L.S.D.		0.250			

Sulfate levels were recorded at the storage period of 0 days as 5.02mg/l, at the 30 days as 4.5mg/l, at the 60 days as 4.05mg/l, and at the 90 days as 3.3mg/l (Table 9). These results are similar to the findings of (Al-sulaiman et al. 2015; Hassan et al. 2021). The So4 was decreased with the increasing storage time from 0-180 days. The results showed significant differences between the sulfate levels at the 0-day and 90-day storage periods (*P*<0.05).

The opposite behavior was recorded in chlorides at the storage period of 0 days as 14.7mg/l, at 30 days as 15.6mg/l, at the 60 days as16.6mg/l, and at the 90 days as 18.12mg/l (Table 10). The results were also compatible with the study of Hassan et al. (2021), where it was found that the chlorides increased with the effect of temperature during storage period. The results o did not show significant differences in the chlorides at the different storage periods. As for potassium levels, they were recorded at 0 days of storage as0.45 mg/l, and decreased to 0.17mg/l at the 90 days (Table 11). The results of sodium (Table 12) and potassium were similar to the findings of Ajekunle et al. (2015) which recorded a decrease in sodium and potassium values with an increase in the time of exposure to the sun during the storage period. The results showed significant differences between potassium levels at the storage stage of 0 and others (*P*<0.05).

Nitrate levels were recorded at the 0-day storage period as 0.62mg/l), at the 30-day as 1.47mg/l, at the 60 days as 2.35mg/L, and at the 90 days as 3.38mg/l (Table 13). 28 days showed an increase in nitrates. The results of the statistical analysis showed that there were significant differences between nitrate levels at storage for 90 days with other storage periods (*P*<0.05).

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