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Monitoring microbiological quality of bottled water as suggested by HACCP methodology

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Abstract

A company, which produces non-carbonated water in 18.9 l bottles, sold to supermarkets through out Crete, Greece, was evaluated. Sampling was carried out in the bottling plant, and at supermarkets where bottled water was stored for 1, 2, and 4 months. In total, 300 samples from final product, 240 samples from raw or filtered water, and 60 samples of empty bottles were analyzed. The results of these analyses indicated the need to improve Hazard Analysis Critical Control Points (HACCP) systems, in order to continuously monitor the water supply source in bottling plants, and to fully implement the correct storage conditions, hygiene procedures, and customer training at supermarkets.

Keywords: Bottled water; Microbial monitoring; Heterotrophic plate count; HACCP

1. Introduction

Bottled water consumption has significantly increased during the last decade (Venieri, Vantarakis, Komninou, & Papapetropoulou, 2006; Warburton, 2000), with an annual growth rate of 25% in North America (Bharath et al., 2003). The European Federation of Bottled Water (EFBW, 2006) estimates the consumption of bottled water in the European Union during 2003 as 45,000 ml (excluding bottles > 10 l) and the consumption in Greece as 59 l per capita. The water-sales worldwide exceed a value of 5 billion euros (Rosenberg, 2003). Non-carbonated bottled water has become more popular than carbonated, being a substitute for tap water in many homes (Armas & Sutherland, 1999). A number of reasons has been reported: consumer awareness about increasing water pollution; deficiencies in municipal water supply in terms of odor, taste, fluoride, chlorine (Papapetropoulou, Tsintzou, & Vantarakis, 1997; Tamagnini & Gonzalez, 1997), as well as successful marketing strategies of the bottling companies (Misund, Frengstad, Siewers, & Reimann, 1999).

Microbial surveys carried out worldwide indicated various problems with bottled water such as: high Heterotrophic Plate Count (HPC) levels (Warburton, Peterkin, Weiss, & Johnston, 1986; Warburton, Dodds, Burke, Johnston, & Laffey, 1992; Warburton, 2000); *Vibrio cholerae* presence and infections (Blake, Rosenberg, Florencia, Costa, & Gangarosa, 1977; Kramer, Herwaldt, Craun, Calderon, & Juranek, 1996); fungal spoilage (Carbal & Fernandez-Pinto, 2002); presence of resistant to antibiotics Pseudomonas species (Guillot & Leclerc, 1993; Hernandez-Duquino & Rosenberg, 1987; Mavridou, 1992); *Staphylococcus aureus* presence (Leclerc, Mossel, & Savage, 1985); *Aeromonas hydrophila* presence (Manaia, Nunes, Morais, & Da Costa, 1990), *Pseudomonas aeruginosa* presence

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(Legnani, Leoni, Rapuano, Turin, & Valenti, 1999); typhoid fever and traveler's-diarrhea reported incidences (Warburton, 1993), etc. Therefore, the bottled water industry has to exhibit strict quality standards in terms of microbial parameters, production processing, bottling, transportation and storage (Cowman & Kelsey, 1992; Hunter, 1993), while Hazard Analysis Critical Control Points (HACCP) systems should be implemented in the bottling process (Venieri et al., 2006).

Bottled water microbiological quality parameters are clearly defined by the Greek laws concerning food standards, in harmonization to the Council of the European Economic Community Directive (1998) 98/83/EEC legislation, according to which: "water should be free from parasites and pathogenic organisms, total coliforms, *Escherichia coli*, *Enterococcus* spp., *P. aeruginosa* should not be detectable in any 250 ml bottled water sample analyzed, while HPC, at 22 °C, and 37 °C, should not exceed 100/ml, and 20/ml CFU, respectively". The 37 °C HPC can provide an indication of fast growing bacteria, related to pathogenic types and the 22 °C HPC an indication of characteristic bacteria that develop slowly (Ramalho, Cunha, Teixeira, & Gibbs, 2001). The aim of this research was to monitor the microbial flora of water in a bottling company during the processing steps, transportation and storage at supermarkets, and to suggest corrective actions in order to maintain microbial quality of bottled water at the retail level.

2. Methodology

2.1. Company description and sampling

This research was carried out during 2005, in the island of Crete, Greece. A specific bottling company was selected, which produces bottled non-carbonated water in 18.9 l, sold through out Crete in supermarkets. This company had implemented the HACCP system and proper procedures were established in place for the observation – monitoring of Critical Control Points (CCPs). Company's bottled water can be used by consumers in homes, or in various companies, or in hospitals; and are connected to autonomous refrigerators, supplied by the same bottling company. Water used for bottling originated from a well protected drill, 300 m deep, pumped into two 20 m^3 stainless-steel water-tanks and undergone three filtration processes (5, 1, and 0.2 μm) before bottling. Each empty bottle and bottle covers were treated with ozonated water before the final bottling. Final products were coded and water bottles were transferred by company's own transportation vehicles to supermarkets.

The sampling process was dived into four sections. During the first section samples were collected at the bottling company: water in (60 samples); water after 5 μm filtration (60 samples); water after 1 μm (60 samples); water after 0.2 μm (60 samples); final product (60 samples); flora in empty water bottles (60 samples) that had been treated with ozonated water. Production codes were recorded as well as the supermarkets where the sampled bottled water was sold. During the other three sections, sampling of final products was carried out at the supermarket level, on products being 1, 2, and 4 months old. Contacts with the supermarkets were arranged in order to secure the availability of bottled water, through out the 4-months evaluation-period; 240 samples were taken from 8 supermarkets, in 4 major towns of Crete, Greece. In total, 300 samples from bottled water, 240 samples from raw or filtered water, and 60 samples of empty bottles were analyzed.

2.2. Microbiological analysis

Microbial water analysis was performed by using the membrane filtration technique (MF) according to International Standards Organization (ISO) protocols, for the detection of total coliforms (TC), (ISO 9308-1:2000, 2000); *E. coli* (EC), (ISO 9308-1:2000, 2000); *Enterococcus* spp. (EN), (ISO 7899-2:2000, 2000); *P. aeruginosa* (PA), (Hellenic Organization for Standardization EN12780:2002); Heterotrophic Plate Count (HPC) at 22 °C, and 37 °C, (ISO 6222:1999, 1999). An advantage of the MF technique is that small numbers of organisms can be detected, because the amount of water passed through the membrane is restricted only by the amount of gross-suspended matter present in water; the MF method permits the analysis of water volumes, ranging from 1 ml to as much as 10 l (Reasoner, 2004). Membrane filter counts may achieve the sensitivity of the multiple tube count, while retaining the accuracy of the colony count method (Reasoner, 2004; Venieri et al., 2006).

Water samples, of 250 ml each, were filtered through hydrophilic mixed cellulose esters membranes (Pall Corporation) of 0.45 μm pore size and 47 mm diameter for all organisms, and of 0.22 μm pore size for *P. aeruginosa* specifically. For the enumeration of HPC, 250 ml of water was filtered and the results were recorded as per ml. Sterile Petri dishes with absorbent pads (Pall Corporation) were filled with 2 ml of ready made sterile ampoule microbiological media: MF-Endo Broth for total coliforms; M-FC Broth with Rosolic Acid for *E. coli*; KF Streptococcal Broth for *Enterococcus* spp.; Pseudomonas broth for *P. aeruginosa*, and M-TGE Broth for HPC (Pall Corporation); membranes were placed in each Petri dish and incubated at 35.5 °C for 24 h, at 44.5 °C for 24 h, at 35.5 °C for 48 h, at 35.5 °C for 24 h and, 22–37 °C for 24–72 h, for each medium and temperature respectively. For the examination of empty water bottles, 250 ml of quarter-strength Ringer's solution, containing 0.05% sodium thiosulphate, was inserted to rinse each bottle, as suggested by Harrigan (1998), then the same filtration and enumeration methodology was used.

3. Results

The microbial characteristics of water inlet to bottling plant, water after the filtration process steps, water as a final product, and empty bottles ready to filled in, are presented

Table 1
Microbial characteristics of water samples at the bottling plant

Microbial parameters	Raw or filtered (processed) water samples								Final product (60 samples)			Empty bottles (60 samples)		
	Water in (60 samples)			Water after 5 μm (60 samples)			Water after 1 μm (60 samples)		Water after 0.2 μm (60 samples)					
	Positive samples[a]	Incidence (%)	Mean (log_{10} CFU/250 ml)	Positive samples[a]	Incidence (%)	Range (log_{10} CFU/250 ml)	Positive samples[a]	Incidence (%)	Range (log_{10} CFU/250 ml)	Positive samples[a]	Incidence (%)	Mean (log_{10} CFU/250 ml)	Positive samples[a]	Incidence (%)	Mean (log_{10} CFU/250 ml)	Range (log_{10} CFU/250 ml)
TC	50	83	2.27	22	37	2.13–2.41	10	17	1.74–1.83	0	–	0.86	0	–	0.96	0.69–1.23	0.56	0.47–0.65
EC	8	13	2.41	4	7	2.20–2.61	4	7	2.04–1.97	0	–	1.04	0	–	1.11	0.95–1.27	0.75	0.60–0.90
EN	0	–		0	–		0	–		0	–		0	–			0	–
PA	0	–		0	–		0	–		0	–		0	–			0	–

TC: Total coliforms, EC: *E. coli*, EN: *Enterococcus* spp., PA: *Pseudomonas aeruginosa*, HPC: Heterotrophic plate count.
[a] Negative were considered the water samples that had TC, EC, EN and PA = 0 CFU/250 ml of water.

in Table 1. Water samples originating from the drill were found with microbial levels greater than 0 CFU/250 ml in terms of total coliforms (TC) (50 samples out of 60) and *E. coli* (EC) (8 samples out of 60), while *Enterococcus* spp. (EN) and *P. aeruginosa* (PA) were found to be 0 CFU/250 ml. Water samples after the 5 μm filtration had smaller levels of TC and EC (20/60, 4/60 CFU/250 ml respectively), which were further decreased (10/60, 4/60 CFU/250 ml) and finally eliminated to 0 CFU/250 ml after the 0.2 μm filtration step, and at the final product.

The Heterotrophic Plate Count (HPC) at 22 °C and 37 °C levels were found to be high in the drill water (all mean log CFU/250 ml 2.27, and 2.41, respectively), and decreased after filtration and bottling; (2.11, and 2.20, respectively, after 5 μm filtration); (1.79, and 2.00, respectively, after 1 μm filtration); (0.86, and 1.04, respectively, after 0.2 μm filtration), and (0.96, and 1.11, respectively, for the final product). Empty bottles were found free from TC, EC, EN, and PA, while Heterotrophic Plate Count (HPC) at 22 °C and 37 °C levels were found to be 0.56, and 0.75, respectively, all mean log CFU/250 ml (Table 1).

During final product storage at supermarkets (1st, 2nd, and 4th month of sampling), total coliforms (TC), E. coli (EC), *Enterococcus* spp. (EN) and *P. aeruginosa* (PA) microbial levels were found to be 0 CFU/250 ml in all 240 samples. The Heterotrophic Plate Count (HPC) at the 22 °C and 37 °C levels were found without significant differences between the eight supermarkets during monthly storage (data not shown), with changes related only to time. Therefore samples are grouped from all eight supermarkets only in terms of storage and presented in Table 2. The HPC levels, at 22 °C, and 37 °C, changed according to time, all mean log CFU/250 ml (0.99, and 1.13, respectively, after 1 month of storage); (1.18, and 1.23, respectively, after 2 months of storage), and (1.23, and 1.26, respectively, after 4 months of storage) (Table 2).

The bottling company had implemented the HACCP system by the time of this research and was certified by an external company. Observation of processing procedures and plant operation, underlined a high degree of compliance to HACCP methodology as well as to Good Manufacturing Practices (GMPs) and Good Hygiene Practices (GHPs), based on: absence of environmental hazards; space availability for maximum production; pest control programs; supplier evaluation; control of raw incoming materials and safe storage; instruction orders; monitoring of Critical Control Points (CCPs); control of working temperatures; final product safe storage; product packaging and labeling; existence of general cleaning and sanitation methods; personnel training and hygiene; equipment maintenance; specifications of raw materials and final products; accreditation methodology for monitoring equipments (e.g. thermometers, pH meters etc); traceability and product recall; management of non conforming products; corrective actions; sampling methods, and internal auditing procedures.

Table 2
Microbial characteristics of water samples during storage at supermarkets

Microbial parameters	Final product (bottled water) stored at supermarkets					
	1st month storage (80 samples)		2nd month storage (80 samples)		4th month storage (80 samples)	
	Mean (log_{10}CFU/250 ml)	Range (log_{10}CFU/250 ml)	Mean (log_{10}CFU/250 ml)	Range (log_{10}CFU/250 ml)	Mean (log_{10}CFU/250 ml)	Range (log_{10}CFU/250 ml)
HPC (22 °C)	0.99	0.95–1.04	1.18	1.15–1.20	1.23	1.18–1.28
HPC (37 °C)	1.13	1.08–1.18	1.23	1.20–1.25	1.26	1.23–1.28

HPC: Heterotrophic plate count.

4. Discussion

The unprocessed water used for bottling by the company studied was found to be inappropriate for human consumption as defined by the 98/83/EEC directive, since it was contaminated with total coliforms (83% incidence) and *E. coli* (13% incidence), even though the outside area of the drill was protected and the water was coming from a deep point (300 m). The main reason for these results most possibly was the animal husbandry activities on the surrounding mountains, contaminating the water well through rain fall and soil permeability, as reported in other cases in the past (Geldreich, 1989). The extension of the bottling plant's HACCP to incorporate the water supply system was the first critical activity taken in order to control the quality of water inlet, as suggested in the literature (Kistemann, Herbst, Dangendorf, & Exner, 2001).

The filtration process (5, 1, and 0.2 μm) succeeded to eliminate the microbial levels of TC and EC to 0 CFU/250 ml and HPC (22 °C and 37 °C) at less than 100/ml and 20/ml, respectively, at the exit of 0.2 μm filter, providing water fully compatible with the 98/83/EEC specifications. The same compatibility was observed in all final product samples, while HPC (22 °C, and 37 °C) was slightly increased, but the final outcome was still within microbiological limits. This increase was possibly due to additional HPC microbes contributed from the empty bottles. Microbial populations can attach to the inner surface of bottles due to cell-surface hydrophobicity of microorganisms, as suggested by Jayasekara, Heard, Cox, and Fleet (1999).

The monthly storage in the supermarkets did not seem to affect the microbial safety of the bottled water, since all the samples tested were within safety specifications, and HPC levels did not exceed 100/ml and 20/ml, at 22 °C and 37 °C, respectively. The contribution of plant's HACCP system was valuable in terms of transportation conditions of the bottled water, since the microbial quality of the final products did not change during the 1st month of storage in supermarkets. The small increase in HPC levels, during the 2nd and 4th month of storage, was within the safety limits with all the tested samples safe for human consumption.

Bottled water can be an oligotrophic environment with sufficient nutrients to maintain autochthonous bacterial growth (Jeena, Deepa, Mujeeb Rahiman, Shanthi, & Hatha, 2006), and growth of bacteria in stored bottles has been reported as a result of specific bottling-materials (Criado, Fernandez-Pinto, Badessari, & Cabral, 2005) that during storage can release organic matter and provide additional substrates for the microbial growth (Evandri, Tucci, & Bolle, 2000). There is also the possibility (Jones, Chamberlain, & Adams, 1999) of pathogen ultramicrocells presence in the raw water, that can pass through the filters, survive and grow slowly in the final product.

On the other hand, studies had shown that bottling water with low HPC initial levels changed very little after seven months of storage (Tsai & Yu, 1997). The results of our research are in agreement with these latter studies since very low HPC levels were recorded, both in the processed and bottled water and in the same bottled water batches at the supermarkets after 1–4 months of storage. Additional reasons that maintained the bottled water quality could be the training sessions that were provided to each supermarket in terms of water safety/hygiene and product storage in a controlled environment (i.e. air-conditioned storage at 20–22 °C).

Concluding, this research underlines the need of introducing and/or expanding the HACCP systems to include the source of water supply in water-bottling plants. The correct implementation of HACCP was recorded to be critical for the microbial quality of bottled water, as well as the transportation conditions, and the storage at the point of retail. A bottling plant that correctly implements the HACCP system, in all processing steps from raw materials to final products, and provides training to personnel at the supermarkets, can control contamination during bottling and prohibit growth during storage and distribution, contributing to public-health safety.

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